NEUTROPHIL-LYMPHOCYTE RATIO AS A MARKER OF DISEASE SEVERITY AND EXACERBATION IN COPD

DISSERTATION SUBMITTED FOR

M.D GENERAL MEDICINE

BRANCH –I

MAY 2018

THE TAMILNADU

DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

CERTIFICATE FROM THE DEAN

This is to certify that this dissertation entitled **"NEUTROPHIL -LYMPHOCYTE RATIO AS A MARKER OF DISEASE SEVERITY AND EXACERBATION IN COPD"** is the bonafide work of **Dr.GOKUL PRASANNAN** in partial fulfillment of the university regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for **M.D General Medicine Branch I** examination to be held in **May 2018.**

Dr. D. MARUTHUPANDIAN M.S, FICS, FAIS
The Honorable Dean
Madurai Medical College
Govt. Rajaji Hospital
Madurai

CERTIFICATE FROM THE HOD

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Dr.V.T.PREM KUMAR, M.D.,
Professor and HOD,
Department Of General Medicine,
Government Rajaji Hospital,
Madurai Medical College,
Madurai.

CERTIFICATE FROM THE GUIDE

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Dr.V.T.PREM KUMAR, M.D.,
Professor and HOD,
Department Of General Medicine,
Government Rajaji Hospital,
Madurai Medical College,
Madurai.

DECLARATION

I, **Dr.GOKUL PRASANNAN,** declare that, I carried out this work on **"NEUTROPHIL-LYMPHOCYTE RATIO AS A MARKER OF DISEASE SEVERITY AND EXACERBATION IN COPD"** at the Department of Medicine, Govt. Rajaji Hospital during the period SEPTEMBER 2016 to SEPTEMBER 2017. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board either in India or abroad.

This is submitted to The Tamilnadu Dr.M.G.R.Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D degree examination in General Medicine.

Place: Madurai **Dr.GOKUL PRASANNAN**

Date:

ACKNOWLEDGEMENTS

At the outset, I wish to thank our Dean **Dr.D.MARUTHUPANDIAN M.S FICS,** for permitting me to use the facilities of Madurai Medical College and Government Rajaji Hospital to conduct this study.

My beloved Head of the Department of Medicine, **Prof.Dr.V.T.PREMKUMAR.M.D**., has always guided me, by example and valuable words of advice and has encouraged innovative thinking and original research work done by post graduates.

I also sincerely thank our beloved professors **Dr.R.BALAJINATHAN, M.D., Dr.M.NATARAJAN, M.D., Dr.G.BAGIALAKSHMI, M.D., Dr.J.SANGUMANI, M.D., Dr.C.DHARMARAJ, M.D., and Dr.R.PRABHAKARAN, M.D** for their par excellence in clinical teaching and constant support.

I am extremely grateful to the Head of the department of Thoracic medicine **Prof. PRABAKARAN MD** for his constant support, guidance, cooperation and encouragement to complete this study.

I would also like to acknowledge the support of Head of the department of Biochemistry, Madurai medical college **Dr. MEENAKUMARI MD** for their laboratory assistance to the study.

I am extremely thankful to the Assistant Professors of Medicine of my unit, **Dr.K.MURALIDHARAN M.D, Dr.S.MURUGESAN M.D,**

Dr.MRAJKUMAR, M.D for their valid guidance, encouragement and suggestions.

My patients, who form the most integral part of the work, were always kind and cooperative. I pray to God give them courage and strength to endure their illness, hope all of them go into complete remission.

I thank my friends and family who have stood by me during my times of need. Their help and support have always been invaluable to me. And last but not the least I would like thank the Lord Almighty for His grace and blessings without which nothing would have been possible.

CONTENTS

INTRODUCTION

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a avoidable and treatable common disease characterized by progressive and permanent airflow limitation associated with increased chronic inflammatory response of the lungs and airways against injurious gases and particles. One of the characteristic features of COPD is acute exacerbations, which usually are associated with increased inflammation due to infections and/or environmental factors. It is possible that bacterial colonization itself, or recurring and intermittent exacerbation caused by the colonization, may contribute to chronic airway inflammation and the progression of COPD. Inflammation is a complex set of relations among various immune-related cells, including neutrophils and lymphocytes, which can lead to persistent tissue damage if targeted destruction and assist repair are not properly phased.

Leucocyte count and its subtypes are well-known markers of inflammation. Since the physiological response of the leucocytes in circulation against stress precipitates an increase in neutrophil count and decrease in the lymphocyte count, the ratio of these two sub-groups to one another is engaged in the intensive care practice. In various recent studies, neutrophil-to-lymphocyte ratio (NLR) has been evaluated for its

probable role in the inflammation periods of chronic diseases such as pancreatitis, acute coronary syndrome etc.

It has been established in previous studies that various inflammatory markers like C-reactive protein (CRP), fibrinogen and leucocyte count increase in stable patients of COPD as well as in exacerbation and that this increase is associated with the negative results of the disease. Unlike other inflammatory biomarkers e.g., ESR and CRP, the Neutrophil–lymphocyte ratio (NLR) is derived from routine complete blood count (CBC) tests. It does not need a special request. It is a rapid, easy and cost-effective method.

The current study is undertaken to analyse the Neutrophil to lymphocyte ratio(NLR) in COPD exacerbation and stable COPD patients. Also to check whether the NLR could reflect the disease severity in such patients as compared with BODE score in COPD.

AIMS AND OBJECTIVES

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- To assess whether Neutrophil-to-Lymphocyte ratio can be used to assess the severity of disease by comparing it with BODE score in COPD
- To assess whether the Neutrophil Lymphocyte ratio is higher in COPD exacerbation compared to patients with stable COPD

REVIEW OF LITERATURE

REVIEW OF LITERATURE

NEUTROPHILS

Neutrophils play imperative part in defence of humans and sometimes it can injure our own tissue. In the resting host,the production and removal of neutrophils are balanced. But at times of stress, chemotactic agents are generated that result in neutrophil recruitment and activation of defense mechanisms.

SUBCELLULAR STRUCTURE

Many types of machinery are within neutrophill when seen by electron microscopy. They are

1. Granules
2. cell membrane
3. Cellular Matrix
4. Layers of Lipid
5. lipid bodies
6. Cytoplasm
7. Multilobed nucleus

Neutrophilic Granules

1. Primary (azurophilic) granules

Antimicrobial enzymes like myeloperoxidase are found in neutrophil, which catalyses the production of hypochlorite through oxidative burst. Other contents include lysozyme, bactericidal permeability increasing protein that alters cell permeability, azurocidin, proteinase 3, esterase and others.

2. Secondary (specific) granules

They are chiefly released into extracellular space and include apolactoferrin, Vit B_{12}-binding protein, plasminogen activator and collagenase. The lack of specific granules predisposes to skin and respiratory infections.

3. Tertiary (Gelatinase) granules

It contains gelatinase, Acetyltansferase, Lysozyme, NRAMP1 and ficolin-1, which are upregulated to the cell surface with stimulation.

4. Secretory Vesicles

Secretory vesicles contain preformed enzymes which are secreted readily stimulation

PLASMA MEMBRANE

It is constituted by ionic channels, ligands, lipid layer for communication in and out of neutrophil.

CYTOSKELETAL MATRIX

Its apparatus include actin, actin binding protein, gelsolin, myosin, tubulin and tropomyosin that help in phagocytosis and exocytosis.

NEUTROPHIL LIPIDS

Lipids account for 5% of neutrophils by weight, of which 35% is phospholipid. Plasma membrane and secretory vesicles contain approximately half of the cellular phospholipid. The presence of arachidonic acid in phospholipids is an important precursor of lipoxins, leukotrienes and prostaglandins.. Non phospholipid neutrophil lipid is chiefly constituted by cholesterol and triglycerides. Glycolipids constitute the remaining

CYTOSOL

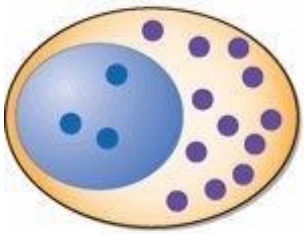
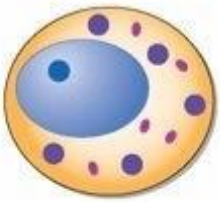
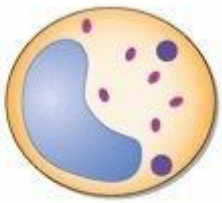
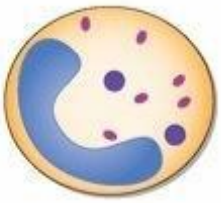
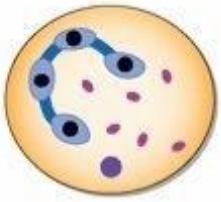
Around 50% of cytosolic protein appears to be migration inhibitory factor related proteins (MRPs). MRP 7 and MRP 13 are members of S 100 family of calcium binding proteins. MRP 13 can result in

macrophage activation. Annexin 1 is the mediator of anti-inflammatory effects of glucocorticoids.

NUCLEUS

Mature neutrophils can produce RNA and protein. This is of particular significance when they migrate to sites of inflammation.

STAGES OF DEVELOPMENT OF NEUTROPHIL.

Cell	Stage	Surface Markers[a]	Characteristics
	MYELOBLAST	CD33, CD13, CD15	Prominent nucleoli
	PROMYELOCYTE	CD33, CD13, CD15	Large cell Primary granules appear
	MYELOCYTE	CD33, CD13, CD15, CD14, CD11b	Secondary granules appear
	METAMYELOCYTE	CD33, CD13, CD15, CD14, CD11b	Kidney bean–shaped nucleus
	BAND FORM	CD33, CD13, CD15, CD14, CD11b CD10, CD16	Condensed, band–shaped nucleus
	NEUTROPHIL	CD33, CD13, CD15, CD14, CD11b CD10, CD16	Condensed, multilobed nucleus

[a]CD= Cluster Determinant; ● Nucleolus; ● Primary granule; • Secondary granule.

PHYSIOLOGIC VARIATION IN NEUTROPHILS

By the age of 4-8 years, the blood differential count approaches that of the adult. Racial variations were reported in black Africans with reduced neutrophil and monocyte and elevated eosinophil counts.. Under conditions of complete physical and mental relaxation, a basal level of 5.0- 7.0 x 10^9 cells/l is usual.Ordinary activity is associated with a moderate increase and a fairly higher level is common in afternoon.

Heat and strong solar radiation are said to cause leukocytosis. Artificially induced heat, sunlight and UV light have been reported to cause lymphocytosis. Acute anoxia, both anoxic and anemic, causes neutrophilic leukocytosis.

Marked leukocytosis occurs regularly with laborious exercise. The increment of cells usually consist of segmented neutrophils, but lymphocytosis may be prominent as well. Such leukocytosis recedes to normal in less than an hour. The magnitude of leukocytosis related with exercise appears to depend primarily on the amount of the activity. Similarly, convulsive seizures are associated with an increase in leukocyte count.

Epinephrine injection produces leukocytosis, the nature and duration of which appear to vary with the mode of administration.

During attacks of paroxysmal tachycardia, leukocytosis with cell counts of 13.0- 22.0 x 10^9 has been reported. Pain, nausea and anxiety may cause leukocytosis. The Paucity of band forms and metamyelocytes indicates that the neutrophilia results from redistribution of cells between marginal and circulating pools.

Many of the physiologic variations in leukocytes that have been described can be explained as manifestations of stimulation of adrenal cortex

REGULATION OF NEUTROPHIL PRODUCTION

The nature of control mechanisms is intricate but several control points exist:

- Recruitment of pluripotent stem cell and making them commitment into committed stem cells,
- Stimulation of stem cell and myeloid proliferative cell growth and
- Selective release of cells from marrow

Several factors that promote neutrophil release from the marrow has been identified including

- C5a,
- TNF alpha,
- TNF beta,
- IL-8 etc

SEQUENCE OF NEUTROPHIL ENDOTHELIAL CELL ADHESION

Tethering ⟶ Rolling ⟶ Adhesion ⟶ Transmigration

A gorgeous model for neutrophil-endothelial cell adhesion was proposed by Springer. According to it, Selectins are responsible for the initial rolling of neutrophils along the endothelial cell. Stimulation of neutrophils can result in a rapid increase in L-selectin affinity for its ligand resulting in tethering of a flowing cell and rolling within a millisecond.

Interaction of chemoattractant molecules on the endothelial surface with ligand receptors of neutrophil result in signal transmission and activation of integrin molecules. These integrins can bind their ligands on the endothelial surface, resulting in a marked increase in adhesion to endothelial cell and cessation of rolling. After this, further chemoattractant molecules are sensed by cells and migrate into the tissues

where neutrophils produce compounds that attract other inflammatory cells.

SELECTINS

Three major selectins have been identified. L-selectin is expressed on the neutrophil surface and its main ligand is a glycoprotein known as *Gly-CAM-1*. Endothelial cells exhibit both E-selectin and P - selectin, both of which recognize Lewis^x-related sialylated carbohydrates. Expression of E-selectin on the vessel surface can be induced with stimuli such as IL-1 and TNF but requires protein synthesis. Thus stimulation of endothelial cells with appropriate stimulus like thrombin or histamine, can effect in a rapid mobilisation of P-selectin to the endothelial cell surface.

INTEGRINS

They are heterodimers of alpha and beta subunits which are connected by non-covalent bond. The important integrins of neutrophil are the *Beta-2* integrins. ICAM 1 expressed on the vascular endothelial cell surface is its ligand. Other Ig superfamily members are also involved, including PECAM-1, ICAM-3 etc

CHEMOTAXIS

Chemotaxis starts with the formation of protrusion known as pseudopodium or lamellopodium at the front of the cell. When the cell started to move, the pseudopodium ruffles rapidly and portion of the pseudopodium attaches to the underlying surface and the contents of the cell move forward into the pseudopodium making the pseudopodium less prominent. This cycle is repeated. In leukocytes, the contraction waveappears to derive in the superficial layer called cortex. Gelsolin plays an important role in neutrophil chemotaxis.

SECRETORY FUNCTIONS OF NEUTROPHIL

A number of substances are released by WBC in vitro. Specific granule release occurs before the primary granule release. Both tertiary granules and secretory vesicles are released even more rapidly. Two modes of enzyme release are, (a) release in to phagocytic vacuoules and (b) true secretion. Lysozyme is present in primary, secondary and tertiary granules and also in monocytes, serum, tear and other secretions.

Stimulated neutrophils can produce and discharge a variety of inflammatory mediators. Neutrophils stimulated with lipopolysaccharides synthesize IL-1, TNF- alpha and IL-1 receptor antagonist, whereas GM-CSF stimulates the production of TNF- alpha and IL-6. On entering the

tissue, neutrophil begin to synthesize cytokines, including the ones that attract other inflammatory cells. In general, diapedesis seems to induce an anti-apoptotic state while phagocytosis promotes apoptosis.

INFLAMMASOMES

IL-1beta is involved in many inflammatory responses. TLR binding to PAMPs result in transcription of Pro-IL-1beta, which is processed by a multimolecular complex termed inflammasome, which converts pro caspase 1 to its active form, which then cleaves Pro-IL-1 beta to IL-1beta. Inflammasomes are present in neutrophils and macrophages. Other neutrophil proteases, including elastase, proteinase 3 and cathepsin G can convert Pro IL-1beta to its active form.

NEUTROPHIL EXTRACELLULAR TRAPS (NETs)

Neutrophils can extrude DNA, histones and granule contents to form structures termed NETs. Microbes can be trapped in these NETs and be killed.

LYMPHOCYTES

Lymphocytes are heterogenous population of cells. They differ greatly regarding there origin, life span, preferred areas of settlement within lymphoid organs. Most lymphocytes are small(10 micrometer or less).Large lymphocytes named as large granular lymphocytes which

contain azurophilic granules in their cytoplasm are also found .Depending on their interaction with correct stromal cells and other growth stimulators, stem cells differentiate into T cells , B cells and other lineages. In thymic microenvironment Tcells develop and with marrow derived stromal cells they develop as B -lymphocytes or myeloid cells. Normal lymphopoiesis depends on Ikaros family of transcription factors.

Although most lymphocytes in normal lymphoid tissue look alike when studied under a microscope, these cells are distinctly divided into two major populations. One of the populations, the T lymphocytes, is responsible for forming the activated lymphocytes that provide cell-mediated immunity, and the other population, the B lymphocytes, is responsible for forming antibodies that provide humoral immunity

Both types of lymphocytes are initially from the embryo started as pluripotent hematopoietic stem cells that form common lymphoid progenitor cells as one of their most important offspring as they differentiate.

Nearly all of the lymphocytes that are formed ultimately end up in the lymphoid tissue, where they are further differentiated or preprocessed in the following ways.

The lymphoid progenitor cells that are destined to eventually form activated T lymphocytes first migrate to and are preprocessed in the thymus gland, and thus they are called T lymphocytes to designate the role of the thymus. They are responsible for cell-mediated immunity.

The other population of lymphocytes-the B lymphocytes that are destined to form antibodies-are preprocessed in the liver during mid-fetal life and in the bone marrow in late fetal life and after birth. This population of cells was first discovered in birds, which have a special preprocessing organ called the *bursa of Fabricius.* For this reason, these lymphocytes are called B lymphocytes to designate the role of the bursa, and they are responsible for humoral immunity

When specific antigens come in contact with T and B lymphocytes in the lymphoid tissue, certain of the T lymphocytes become activated to form activated T cells, and certain of the B lymphocytes become activated to form antibodies. The activated T cells and antibodies in turn react highly specifically against the particular types of antigens that initiated their development. The mechanism of this specificity is the following.

Millions of Specific Types of Lymphocytes Are stored in the Lymphoid Tissue .Millions of different types of preformed B lymphocytes and preformed T lymphocytes that are competent of forming

highly specific types of antibodies or T cells have been stored in the lymph tissue, as explained prior. Every preformed lymphocytes has the capacity to form only one type of antibody or one type of T cell with a single type of specificity. And only the corresponding type of antigen which it can bind with can activate it. After the specific lymphocyte is activated by its antigen, it multiplies wildly, forming large numbers of similar lymphocytes.

- If it is a B lymphocyte, its progeny will eventually secrete the specific type of antibody that then circulates throughout the body.
- If it is a T lymphocyte, its progeny are specific sensitized T cells that are released into the lymph and then carried to the blood and circulated through all the tissue fluids and back into the lymph, sometimes circulating around and around in this circuit for months or years.

Neutrophils comprise 40-75% (1800-7700/l) and lymphocytes 20-50% (1000-4800/l) of the total leucocyte count

LYMPHOCYTOSIS is said to occur when there is an increase in absolute counts of lymphocytes. It should not be applied to an increase in relative proportion of lymphocytes in the absence of an absolute increase in lymphocyte count.

Causes of Lymphocytosis

1. Pertusis
2. Typhoid
3. Tuberculosis
4. Secondary Syphilis
5. Tropical Splenomegaly Syndrome
6. Epstein Barr Virus Infection,
7. Cytomegalovirus
8. Leukemias are some causes of lymphocytosis.

LYMPHOPENIA is said to occur when the absolute lymphocyte count is below the conventionally employed lower limit of normal 1.5 *10^9.

Lymphopenia is common in leucopenic prodromal phase of many viral infections, pancytopenia due to any cause, steroid intake and many more.

Inflammation is stirred by chemical mediators that are produced in host cells in response to deleterious injury or stimuli. When cardiac tissue is injured, the presence of damage is sensed by resident cells mainly macrophages, neutrophils & lymphocyte and also dendritic cells, mast cells, and other cell types. These cells secrete molecules (cytokines and

other mediators) that induce and regulate the subsequent inflammatory response.

Inflammatory mediators are also produced from plasma proteins that react with the injured tissues. Some of these mediators stimulate the efflux of plasma and the enrolment of circulating leukocytes to the inflammatory site where the offending tissue is located.

All these recruited leucocytes are activated and they try to remove the offending tissue by phagocytosis.

An unfortunate side effect of the activation of leukocytes is that it may cause injury to normal host cells also. Acute inflammatory process occurring as a result of an insult to a tissue or organ has two major components:

Vascular changes

Changes in vessel caliber causes an augmented blood flow (vasodilation) and alterations in the vessel wall that allows plasma proteins like albumin to leave the intravascular circulation (increased vascular permeability). In addition, endothelial cells are activated, resulting in increased adhesion of neutrophils, lymphocytes along with macrophages and relocation of these agents through the vessel wall.

Cellular events

Migration of the leukocytes from the circulation and gathering in the focus of injury (cellular recruitment), followed by activation of the leukocytes (neutrophils, lymphocytes, macrophages) enabling them to do the repair or to remove the necrotic debri. The principal leukocytes in acute inflammation are neutrophils (polymorphonuclear leukocytes). Multiple mechanisms may lead to transient or permanent increase in vascular permeability in acute inflammatory reactions:

1. Vascular Endothelial cell contraction that forms intercellular gaps in post capillary venules is the most common cause of increased vascular permeability.
2. Endothelial injury results in vascular leakage by causing endothelial cell necrosis and detachment
3. Increased transcytosis of proteins by way of an intracellular vesicular pathway augments venular permeability after exposure to particular mediators like vascular endothelial growth factor (VEGF)
4. Leakage from new blood vessels.

Leukocyte Recruitment

Leukocytes usually flow rapidly in the blood, and in inflammation, they have to be stopped and brought to the the site of tissue damage, which are usually outside the vessels. The sequence of events in the recruitment of leukocytes from the vascular lumen to the extravascular space consists of

a) Margination and rolling along the vessel wall.

b) Firm adhesion to the endothelium.

c) Transmigration between endothelial cells

d) Migration in interstitial tissues toward a chemotactic stimulus.

Rolling, adhesion, and transmigration are mediated by the interactions of adhesion molecules on leukocytes and endothelial surfaces.

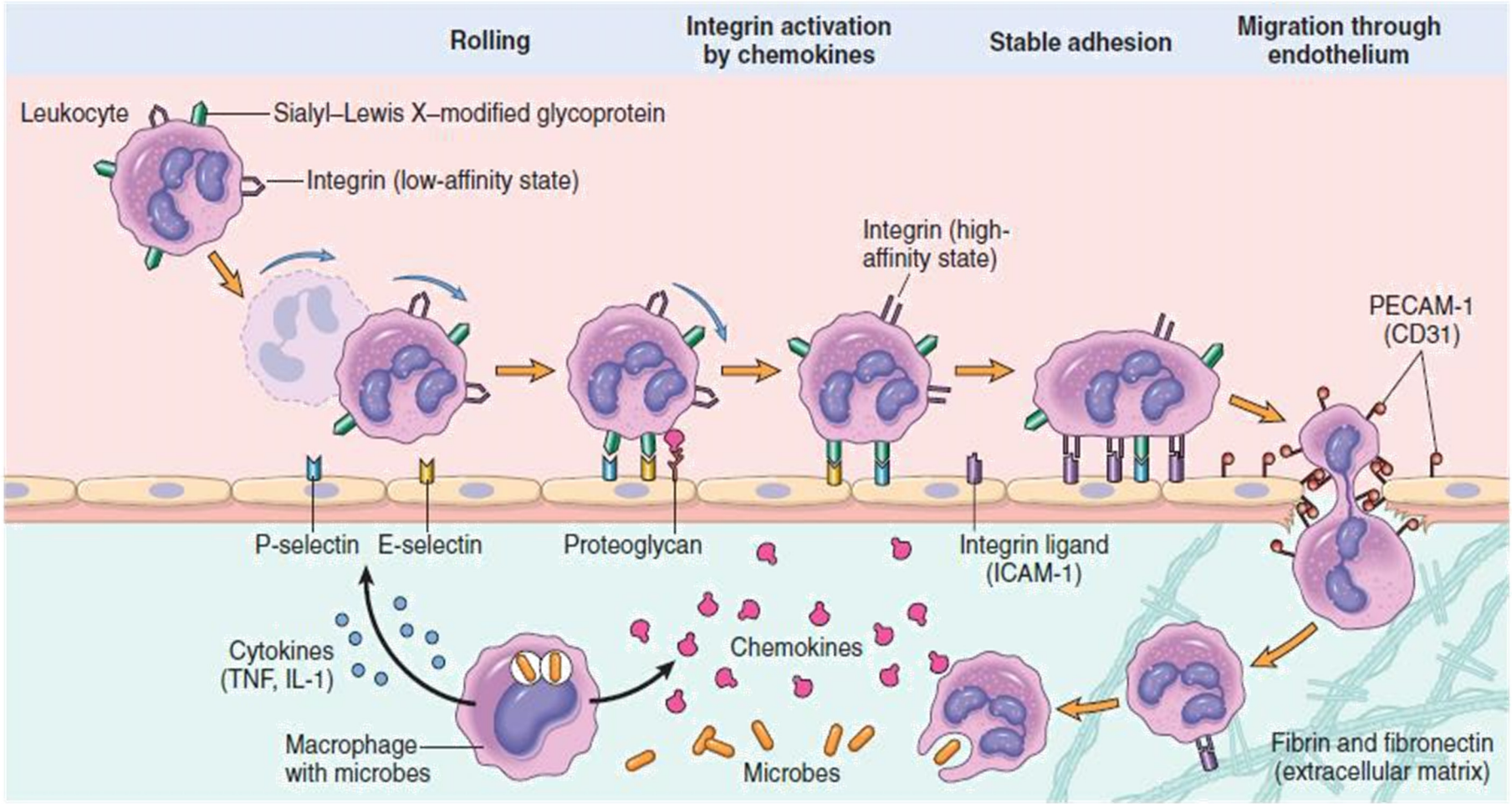
Chemical mediators which act as chemoattractants and certain cytokines can affect these processes by modulating the surface expression and binding properties of leucocytes.

Mechanisms of leukocyte migration through blood vessels

The leukocytes (neutrophils shown here) first roll, then become activated and hold on to endothelium, and then transmigrate across the endothelium, pierce the basement membrane, and migrate toward chemoattractants emanating from the source of injury.

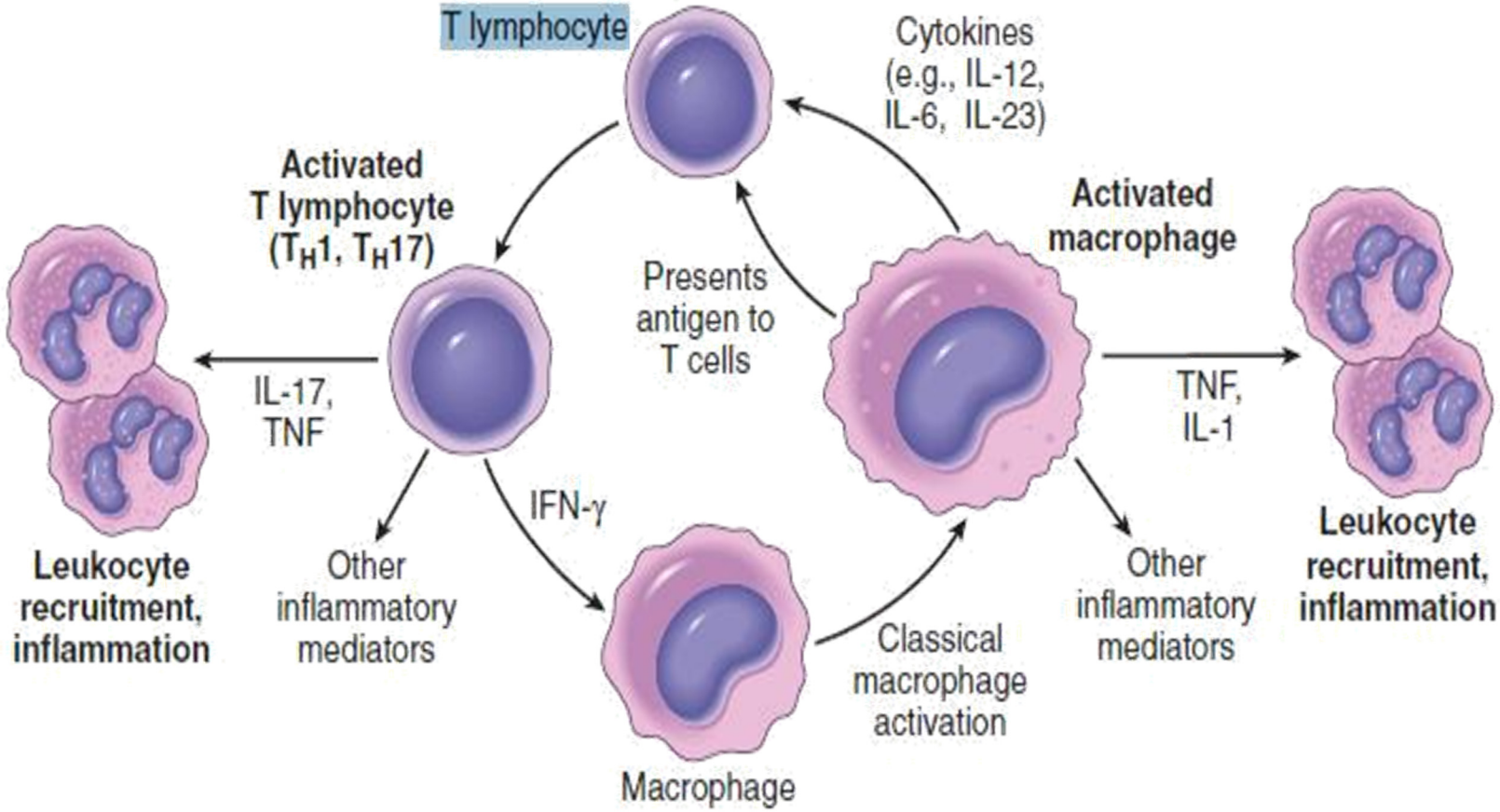
Different molecules play predominant roles in different steps of this process: selectins in rolling. chemokines (usually displayed bound to proteoglycans) in activating the neutrophils to increase avidity of integrins. The various chemo attractants and binding receptors are

- Integrins in firm adhesion and
- CD31 (PECAM-1) in transmigration.
- ICAM-1, intercellular adhesion molecule-1;
- IL-1, interleukin-1;
- PECAM-1, platelet endothelial cell adhesion molecule-1;
- TNF, tumor necrosis factor.


Rolling
Integrin activation by chemokines
Stable adhesion
Migration through endothelium
Leukocyte
Sialyl–Lewis X–modified glycoprotein
Integrin (low-affinity state)
Integrin (high-affinity state)
PECAM-1 (CD31)
P-selectin
E-selectin
Proteoglycan
Integrin ligand (ICAM-1)
Cytokines (TNF, IL-1)
Chemokines
Macrophage with microbes
Microbes
Fibrin and fibronectin (extracellular matrix)

In most forms of acute inflammation, neutrophils dominate in the inflammatory infiltrate during the first 6 to 24 hours and are replaced by monocytes in 24 to 48 hours .Several factors account for this early abundance of neutrophils: These cells are the most numerous leukocytes in the blood, they respond more rapidly to chemokines, and they may attach more firmly to the adhesion molecules that are rapidly induced on endothelial cells. But are short-lived—they die by apoptosis and disappear within 24 to 48 hours—while monocytes survive longer. Because leukocytes are capable of secreting potentially harmful substances such as enzymes and reactive oxygen, they are important causes of injury to normal cells and tissues under several circumstances .For example, after a myocardial infarction as an effort to clear injured and dead tissues in an infarct, inflammation may extend and aggravate the injurious consequences of the ischemia, especially on reperfusion.

Lymphocytes are mobilized in the setting of any specific immune stimulus (i.e., infections) as well as non–immune mediated inflammation (e.g., due to ischemic necrosis), and are the major drivers of inflammation in many chronic inflammatory diseases.


T lymphocyte
Cytokines
(e.g., IL-12,
IL-6, IL-23)
Activated
T lymphocyte
(T_H1, T_H17)
Activated
macrophage
Presents
antigen to
T cells
IL-17,
TNF
TNF,
IL-1
IFN-γ
Leukocyte
recruitment,
inflammation
Other
inflammatory
mediators
Classical
macrophage
activation
Other
inflammatory
mediators
Leukocyte
recruitment,
inflammation
Macrophage

By secreting cytokines, CD4+ T lymphocytes promote inflammation and alters the course of the inflammatory reaction. There are 3 types of CD4+ helper T cells. They secrete different types of cytokines and produce different types of inflammation:

1. **TH1** cells secrete the cytokine IFN-γ that alters inflammation by activating macrophages, thus stimulating classical complement pathway
2. **TH2** cells secrete IL-5, IL-13, and IL-4, which recruit and stimulate eosinophils, thus activating alternate complement pathway.
3. **TH17** cells secrete IL-17 and other cytokines that induce the secretion of chemokines responsible for recruiting neutrophils and monocytes into the reaction.
4. Activated lymphocytes and macrophages stimulate each other, and both cell types release inflammatory mediators that affect other cells.

NEUTROPHIL-LYMPHOCYTE RATIO

There has been a recent focus on differential WBC count in predicting cardiovascular risk of the many cell types used like neutrophil count, lymphocyte count, monocyte count, neutrophil lymphocyte ratio (NLR) is found to have predictive superiority. Elevated neutrophil level and decreased lymphocytes are independent predictors of poor outcome in COPD and various other cardiac conditions but NLR ratio is superior to either of these alone Reasons for its superiority include:

1. Though physiological conditions like dehydration and exercise might alter the absolute number of individual cell types, the ratio remains less affected
2. NLR is a ratio of two different complementary immune pathways. Neutrophils are involved in quicker response while lymphocytes are related to more adaptive long term response.

Integrated reflection of two important and opposite immune pathways is more dependable than either alone. NLR is cheap, easily assesable and readily available marker that can help in risk stratification of patients with various diseases .Elevated NLR is associated with arterial stiffness & progression of atherosclerosis. An increased neutrophil count might reflect inflammation and lymphopenia is an indicator of physiologic stress due to increased levels of corticosteroids & lymphocyte apoptosis.

Neutrophils produce the enzyme myeloperoxidase that is involved in promoting phagocytic function of neutrophils, but high levels of this enzyme can cause excess production of free radicals which is responsible for tissue injury.

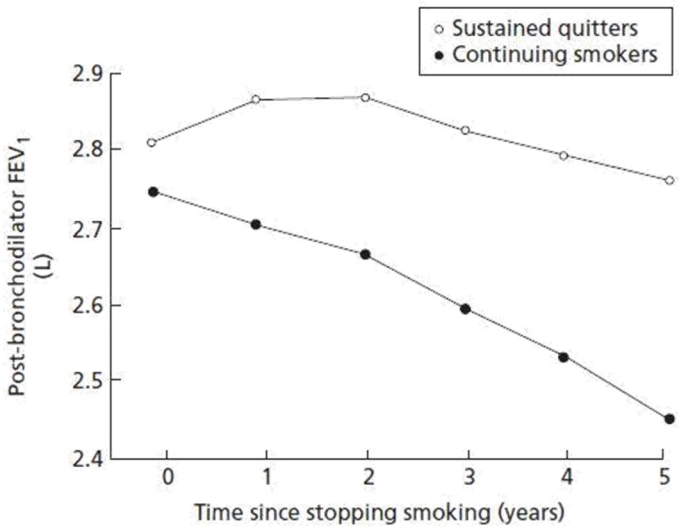
Biomarkers are now being more and more used in diagnosing various conditions and also for determining short and long term prognosis. Role of biomarkers in our clinical practice is expected to grow tremendously in coming years. Inexpensive and readily available biomarkers like NLR will be of great use in assessing prognosis of patients admitted with various conditions like COPD

Chronic Obstructive Pulmonary Disease [COPD]

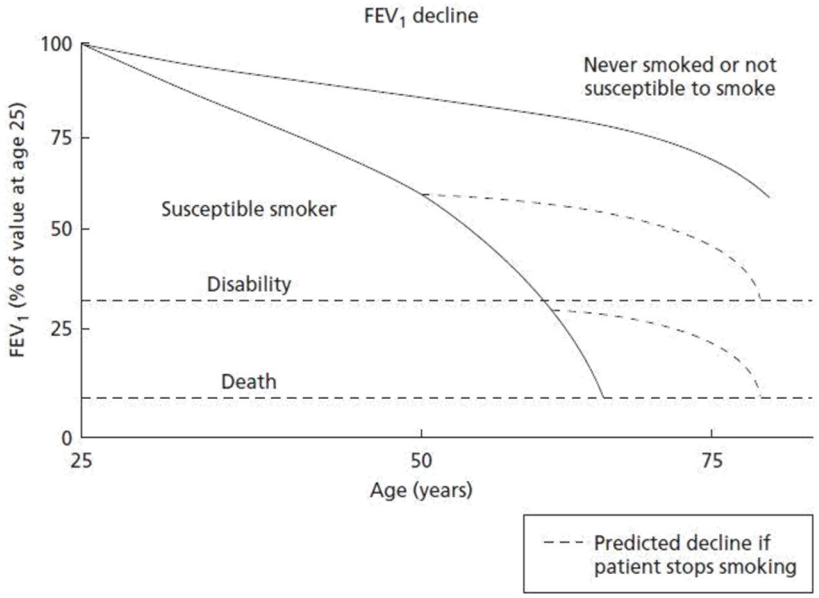
COPD has been defined as "a disease state characterised by airflow limitation that is not fully reversible." COPD includes *emphysema*, anatomically defined by the destruction and enlargement of distal airspaces; *chronic bronchitis*, clinically defined by chronic cough and sputum; and *small airways disease* causing bronchiolar narrowing.

RISK FACTORS

1. Cigarette smoking is definitively the most important individual causative factor of COPD. However, only 20% of smokers go on to develop clinically significant disease. Pack years of smoking is the most highly significant predictor of FEV1.



The above table depicts the effect of age on airflow obstruction in normal subjects and in susceptible cigarette smokers. Quitting the smoking habit will return the rate of decline to the normal trend.

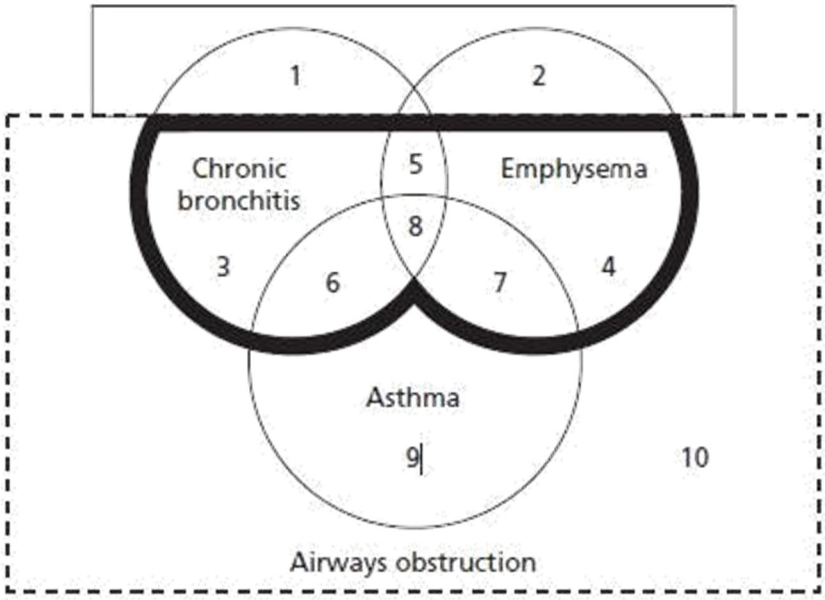


The above chart depicts the mean FEV1 after bronchodilator administration in ex-smokers who maintained abstinence compared with smokers who continued to smoke.

2. Airway hyperresponsiveness, one of the characteristic features of bronchial asthma, is seen in many patients with COPD.

Overlap between asthma and COPD was the basis for the development of Dutch hypothesis which proposes that asthma, emphysema and chronic bronchitis are variations of the same disease, altered by genetics and environment to produce these phenotypes.

The British hypothesis proposes that asthma is completely diverse from COPD-asthma being an allergic phenomenon and COPD the sequel of smoking related inflammation and damage.



A non-proportional Venn diagram telling the relation between asthma, emphysema and chronic bronchitis/ the broken line rectangle includes all patients with airflow obstruction. Patients in subsets 1 and 2 have clinical or radiological features of chronic bronchitis or emphysema but do not have airflow obstruction and thus have a normal FEV1 and FEV1/FVC ratio. These patients are not classified as having COPD. Patients in subsets 6-8 have partially reversible airflow obstruction. Subsets 3-5 have no significant reversibility and patients in subset 8 have features of all three disorders. Those in subset 9 have completely reversible airflow obstruction and thus classified as asthma. Those in subset 10 have airflow obstruction due to specific causes like cystic fibrosis, bronchiectasis. Patients with COPD are those within the thick shaded band.

3. Ambient air pollution is a much less significant risk factor than cigarette smoking.

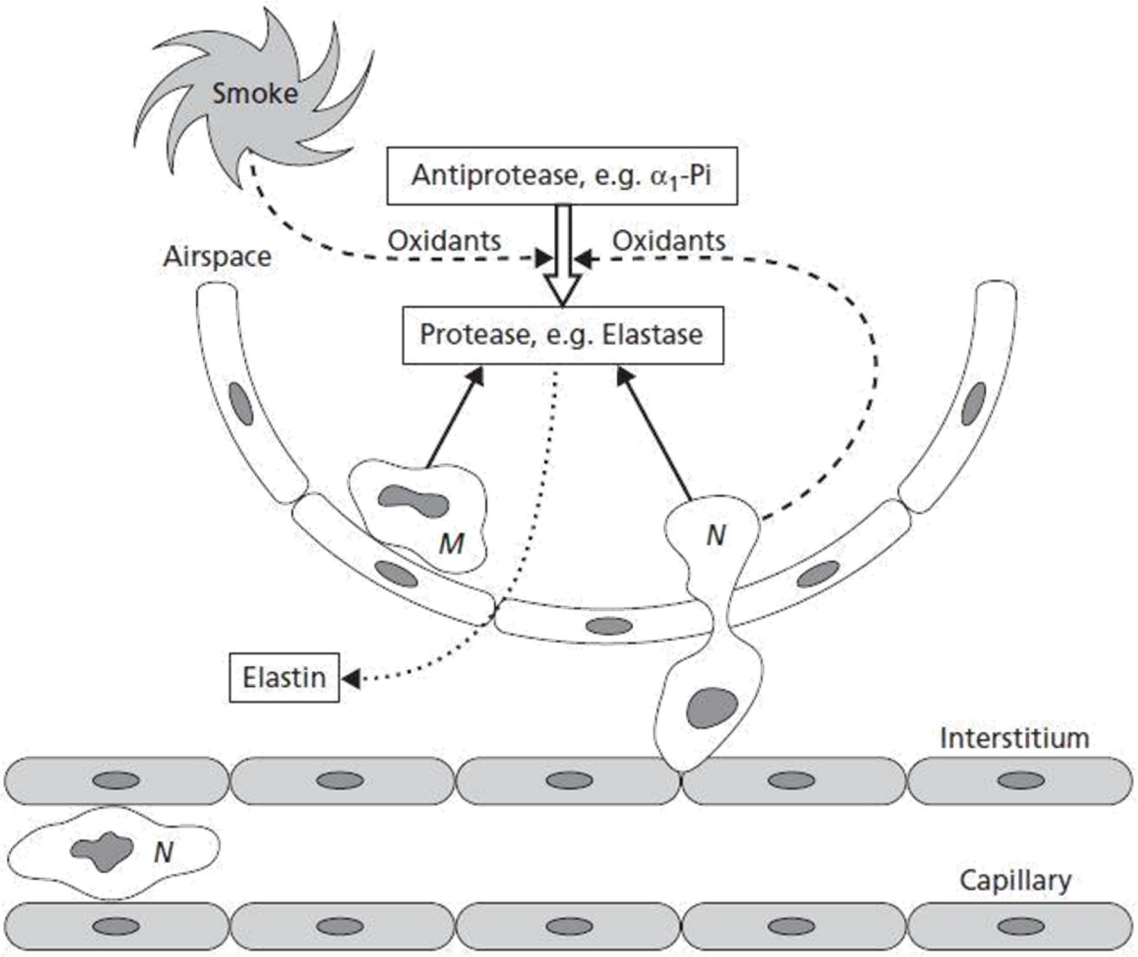
4. Passive smoking exposure has been linked to decreased lung function, but relation with COPD remains unproven

PATHOGENESIS OF COPD

Both chronic bronchitis and emphysema produce airway narrowing but evidence of obstruction need not be present. Airway obstruction is always seen by the time the patient becomes dyspnoeic. Airflow limitation, which is the major physiological change in COPD, can result from both small airway obstruction and emphysema.

PROTEASE- ANTIPROTEASE THEORY

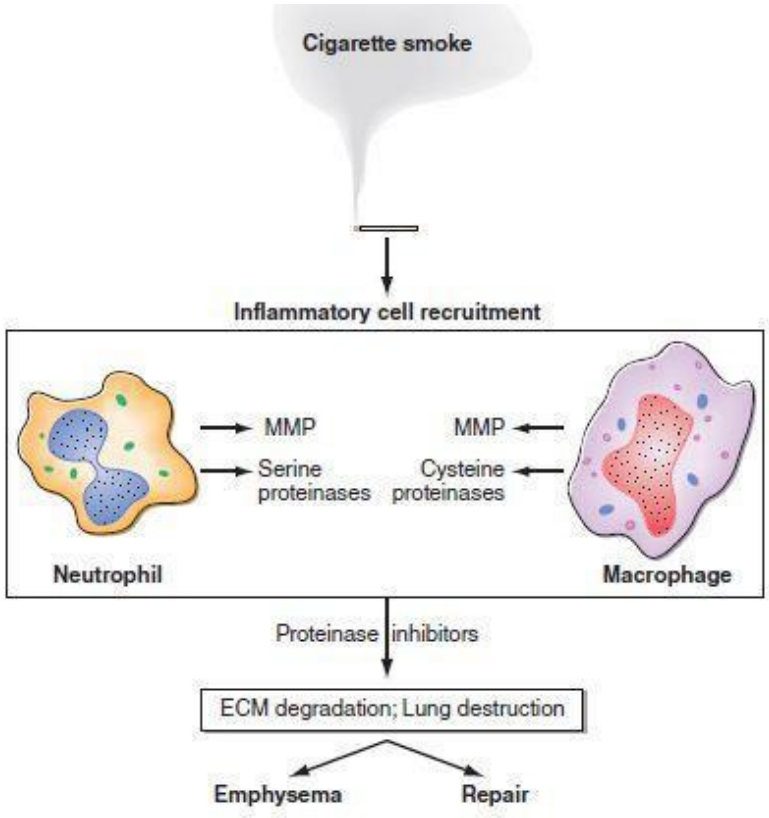
Cigarette smoke has a pro- oxidant effect, which makes neutrophils less deformable and causes neutrophil sequestration in the pulmonary capillaries. Activated neutrophils initially adhere to the endothelium and then migrate to the airspaces. The pro-oxidants from cigarette smoke or released by activated airspace neutrophils inactivate antiproteases, mainly elastase. Elastase enters the lung interstitium and destroys elastin, causing destruction and enlargement of distal airspaces.


Smoke
Antiprotease, e.g. α_1-Pi
Oxidants
Oxidants
Airspace
Protease, e.g. Elastase
M
N
Elastin
Interstitium
N
Capillary

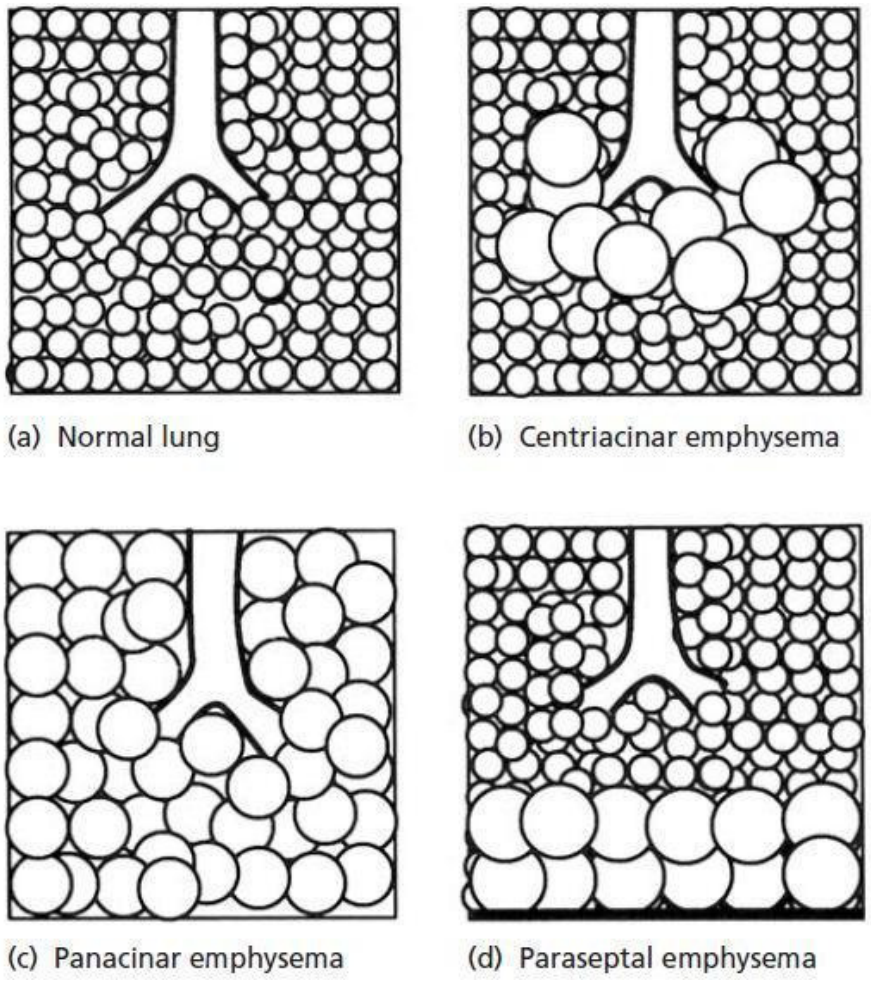
INFLAMMATION AND EXTRACELLULAR MATRIX HYPOTHESIS

Macrophages and epithelial cells are activated on exposure to oxidants in cigarette smoke and produce chemokines that attract inflammatory cells, for example matrix metalloproteinases, IL-8 and TNF which lead to neutrophil recruitment.CD8+T cells are also recruited and release interferon inducible protein-10 (IP-10) which simulate macrophage production of macrophage elastase- matrix metalloproteinase-12.

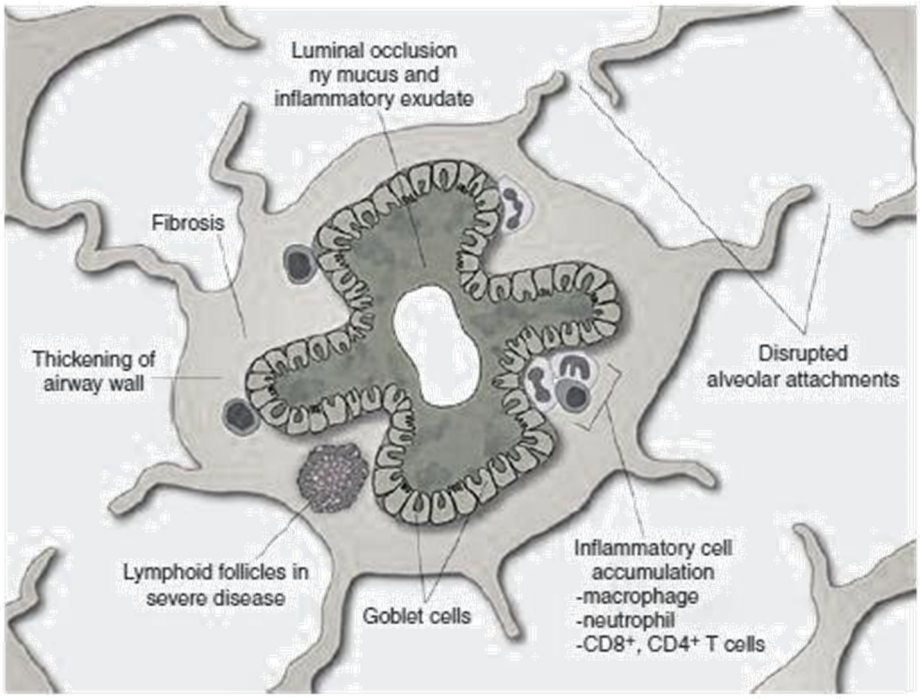
Matrix metalloproteinases and serine proteinases (especially neutrophil elastase) work synergistically by degrading the other's inhibitor and produce lung destruction.



TYPES OF EMPHYSEMA



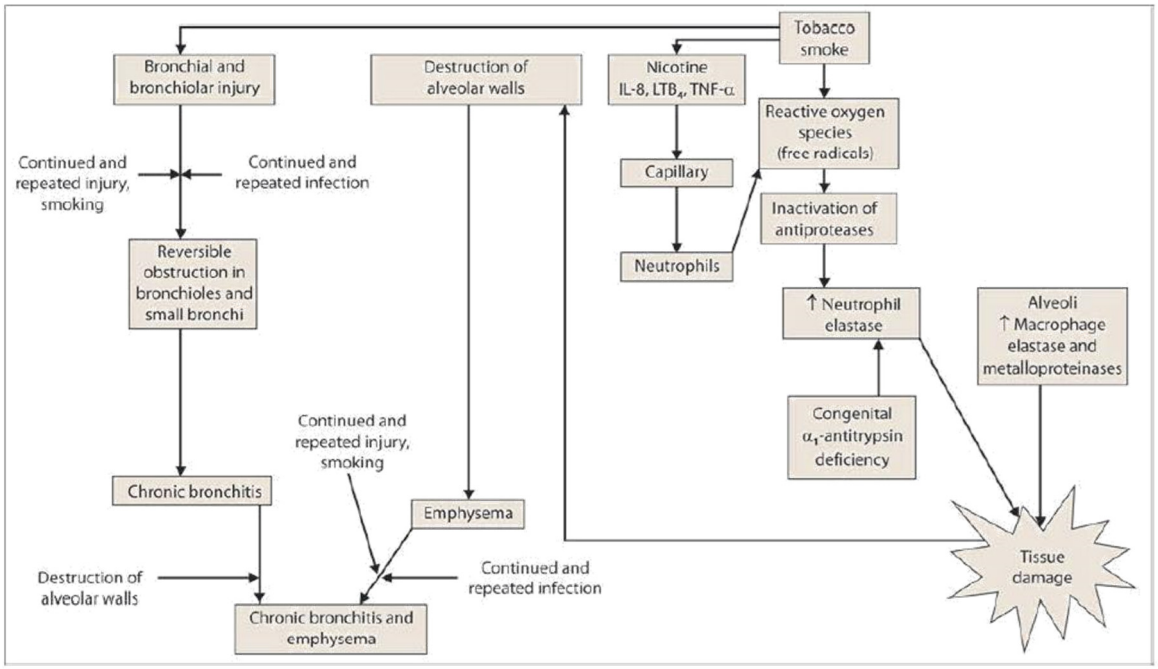
- **Centriacinar**- destruction of central acinus, commonly affect upper lobes, common in smokers
- **Panacinar**- widespread destruction of acinus, commonly affect lower zones, common in antitrypsin deficiency
- **Paraseptal**- affects the distal acinus, can cause spontaneous pneumothorax



The above picture demonstrates the multiple individual pathologies ultimately leading to small airway disease with reduction of FEV1.

CELL DEATH AND INEFFECTIVE REPAIR

Structural cell death caused by oxidants in cigarette smoke occurs through a number of mechanisms, including rt801 inhibition of mammalian target of rapamycin (mTOR), leading to cell death and proteolysis. Involvement of mTOR and other senescence indicators has funded recent theories that emphysema is a type of premature accelerated ageing of lung. Macrophage uptake of apoptotic cells is inhibited by cigarette smoke, which limits repair.



PATHOPHYSIOLOGY OF COPD

Persistent reduction in forced expiratory flow rates is the most classical finding in COPD.

AIRFLOW OBSTRUCTION

Also known as airflow limitation, it is assessed by spirometry, by forced expiratory maneuvers after patient inhales to total lung capacity. Most essential measurements are the volume of air exhaled in the first second of the forced expiratory maneuver (FEV1) and the total volume of air exhaled during the complete spirometric maneuver [Forced Vital Capacity (FVC)]. Airflow obstruction of COPD is identified by a persistently reduced FEV1/FVC ratio. Unlike bronchial asthma, the

decreased FEV1 in COPD does not show improvements more than 15% in response to inhaled bronchodilators.

The airflow in forced exhalation depends upon the balance between elastic recoil of the lungs which assists flow and the airway resistance inhibiting flow. In COPD, maximal expiratory flow decreases as the lung empties because the parenchyma gives progressively less recoil and the airflow resistance increases due to decrease in airway cross sectional area.

HYPERINFLATION

Pulmonary function tests also measure lung volumes. “Air trapping” (increased residual volume and increased ratio of residual volume to total lung capacity) and increasing hyperinflation (increased total lung capacity) occurs in COPD. Hyperinflation is an initial compensatory mechanism which helps in maintaining maximal expiratory airflow, as the increased lung volume increases the elastic recoil and the airways enlarge decreasing the airway resistance.

However, the flattening of diaphragm due to hyperinflation of lungs is not good for the COPD patient:

1. As diaphragm and abdominal wall are now closely apposed, positive abdominal pressure in inspiration cannot be as effectively delivered to chest, which decreases rib cage movement and reduces inspiration.

2. The muscle fibres of the flattened diaph*r*agm are shorter than normal and hence, not able to produce normal inspiratory pressures.

3. Flat diaphragm has more radius of curvature ***r***, hence, increased tension ***t*** must be produced to maintain transpulmonary pressure ***p*** for tidal breathing-{LAPLACE LAW p=2t/r}

4. Inspiratory muscles must overwork to overcome the resistance of the expanded thoracic cage.

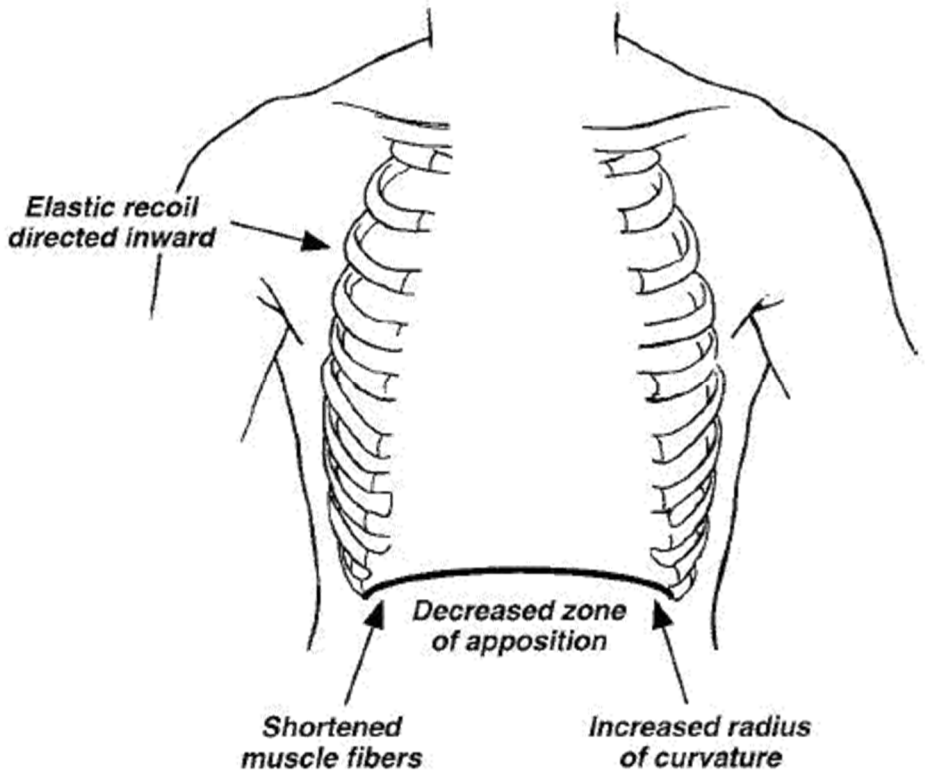


Diagram depicting the mechanisms by which flat diaphragm eventually contributes to COPD mortality

GAS EXCHANGE

Partial pressure of oxygen in arterial blood (PaO2) remains normal until FEV1 decreases to less than 50% of predicted. Increased PaCO2 occurs only when FEV1 decreases to less than 25% of predicted.

Pulmonary hypertension causing cor pulmonale and right ventricular failure occurs when FEV1 decreases to less than 25% of predicted with chronic hypoxemia (PaO2 < 55mm Hg).

Mechanisms of development of Pulmonary Hypertension in COPD

- Degeneration of the vascular bed in lungs
- Alterations in blood gas tension
- Alterations in lung mechanics
- Elevated cardiac output
- Alterations in blood volume
- Increased velocity of blood
- Changes in lung endothelium

Systemic Features of COPD

Systemic features	*Possible mechanism*
Cachexia	TNF-α, IL-6, leptin
Muscle wasting	Apoptosis of skeletal muscle due to TNF-α
Polycythaemia	Chronic hypoxia
Anaemia	TNF-α
Depression	TNF-α, IL-6
Cardiovascular abnormalities	CRP, fibrinogen
Osteoporosis	? effect of corticosteroid therapy

Conditions suggesting alpha-1 anti-trypsin deficiency
Early-onset emphysema (age under 45 years)
Emphysema in a nonsmoker
Emphysema predominantly in lung bases (pan-acinar)
Necrotizing panniculitis (Weber-Christian disease)
c-ANCA positive vasculitis (e.g., Wegener's granulomatosis)
Family history of early onset emphysema or non-smoking–related emphysema
Bronchiectasis without other etiology

DIAGNOSIS

COPD must be thought of in patients with chronic complaints of cough, sputum production or dyspnea with history of smoking and exposure to risk factors.

SEVERITY SCORES FOR RESPIRATORY DISEASES

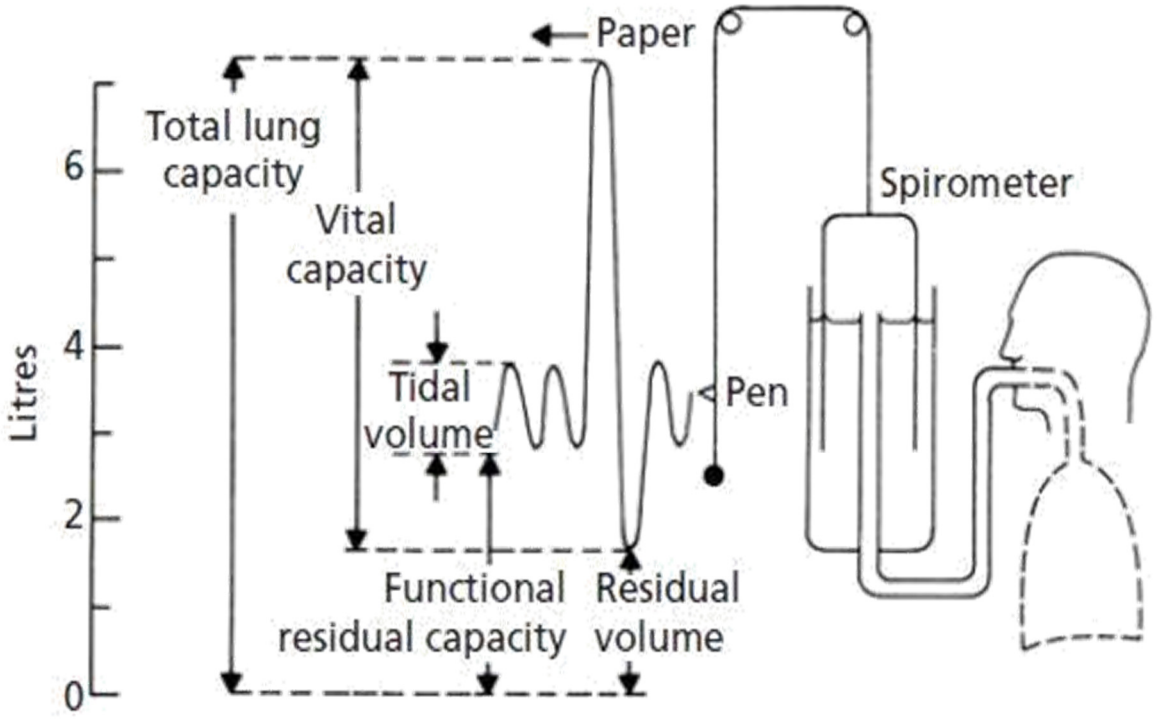
Calculation of the BODE Index*				
	Points on the BODE Index			
Variable	0	1	2	3
FEV_1 (% predicted)	≥65	50–64	36–49	≤35
Distance walked in 6 min (meters)	≥350	250–349	150–249	≤149
MMRC dyspnea scale	0–1	2	3	4
Body-mass index (kg/M^2)	> 21	≥21		

A patient with a BODE score of 0-2 has a mortality rate of around 10% at 52 months,whereas a patient with a BODE score of 7-10 has a mortality rate of around 80% at 52 months.

MMRC Dyspnoea Scale

Modified Medical Research Council Dyspnea Scale (MMRC Scale)	
Grade	**Description**
0	Not troubled with breathlessness except with strenuous exercise
1	Troubled by shortness of breath when hurrying on the level or walking up a slight hill
2	Walks slower than people of the same age on the level because of breathlessness or has to stop for breath when walking at own pace on the level
3	Stops for breath after walking about 100 yards or after a few minutes on the level
4	Too breathless to leave the house or breathless when dressing or undressing

Spirometry



A post bronchodilator FEV1/ FVC < 0.7 confirms airway obstruction that is not completely reversible.

GOLD CRITERIA FOR COPD

GOLD Stage	SEVERITY	SPIROMETRY
I	Mild	FEV1/FVC<0.7 and FEV1 >80% predicted
II	Moderate	FEV1/FVC<0.7 and FEV1 >50% but <80% predicted
III	Severe	FEV1/FVC<0.7 and FEV1 >30% but <50% predicted
IV	Very Severe	FEV1/FVC<0.7 and FEV1 <30% predicted

IMAGING

Chest X-ray may be normal or show emphysematous changes. It is very helpful in ruling out other differential diagnoses and in detecting complications of COPD, including life threatening ones like pneumothorax. Patients with chronic bronchitis may have thick bronchial walls which appear as tubular or tram track shadows with increased vascular markings.

Chest X-ray in symptomatic emphysematous patients reveals dark hyperlucent lung fields with decreased vascular markings, characteristic bullae, flattened and pushed-down diaphragm and tube-like heart.

HRCT can readily detect emphysema but is not used routinely for the purpose of diagnosis. Contrast-enhanced computed tomography (CECT) chest may show a dilated pulmonary artery, indicating pulmonary hypertension.

ACUTE EXACERBATION

An exacerbation is defined as an episode of increased cough, dyspnea and altered volume and character of sputum, with or without other signs of disease. The frequency of exacerbations greatly affects quality of life of COPD patients, especially those with GOLD stage III or IV, who experience one to four exacerbations in a year.

Chances of exacerbation in future are increased by a previous history of exacerbation and an increased ratio of pulmonary artery diameter to aorta diameter on Chest CT.

Bacterial infection causes more than half of exacerbations, with viral infections being involved in 30% and remaining 20 % cases having no obvious precipitating cause.

Prevention of exacerbations is achieved to a great degree of success with inhaled steroids, anticholinergics and long acting beta agonists.

Treatment is by inhaled beta agonists, along with anticholinergic agent, antibiotics, oral glucocorticoids and supplemental O2.

MATERIALS AND METHODS

MATERIALS AND METHODS

TITLE

NEUTROPHIL LYMPHOCYTE RATIO AS A MARKER OF DISEASE SEVERITY AND EXACERBATION IN COPD

STUDY DESIGN

Analytical Cross-sectional study

STUDY PERIOD

SEPTEMBER 2016 to SEPTEMBER 2017(1 year)

SELECTION OF STUDY SUBJECTS

The study is to be conducted among 80 patients which includes 30 patients who attend the emergency department of Gov.Rajaji Hospital, Madurai in a state of COPD Acute exacerbation and 50 Stable COPD patients who attend the OPD for followup.

Criteria for COPD Exacerbation- any patient with a worsening of more than two respiratory symptoms (dyspnoea,sputum,cough or wheeze) for two or more consecutive days can be considered as exacerbation.

Criteria for stable COPD-no symptoms of exacerbation and no use of systemic corticosteroids or antibiotics for the preceding 8 weeks.

INCLUSION CRITERIA

All patients with a confirmed diagnosis of COPD diagnosed with pulmonary function test according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria

EXCLUSION CRITERIA:

- Bronchial asthma, bronchiectasis, or bullous lung disease
- Active tuberculosis or any history of pulmonary fibrosis
- Any tumor, hepatitis, thyroid diseases, autoimmune diseases, or any other acute infections
- Dementia
- Receiving systemic corticosteroids, antibiotics, or immunosuppressive treatment
- Not free from an Exacerbation for at least preceeding 8 weeks
- Withdrawal of consent

ANTICIPATED OUTCOME

Neutrophil-to-lymphocyte ratio is expected to be elevated in COPD patients and is a marker of disease severity in stable COPD patients in comparison with BODE score. NLR value is expected to increase during acute exacerbation.

METHOD OF COLLECTION OF DATA

Patients with acute exacerbation of COPD brought to the Medicine casualty and also stable COPD patients attending the Thoracic Medicine OPD of Gov.Rajaji Hospital, Madurai.

Informed consent was taken

A detailed history including duration of illness, smoking history, pack-years and any past medical history was taken.

Degree of dyspnoea was assessed using Modified Medical Research Council (MMRC) dyspnoea scale

Body mass index calculated.

Pulmonary function tests were done including FEV1/FVC and Post-bronchodilator FEV1

Peripheral blood samples were collected from all patients within 24hours of admission which includes total count, differential count of neutrophils and lymphocytes.

Neutrophil-to-lymphocyte ratio was calculated by dividing absolute neutrophil count by absolute lymphocyte count

LABORATORY INVESTIGATIONS

Total leucocyte count

Differential count of neutrophils and lymphocytes,

COLLABORATING DEPARTMENTS

Department of General medicine

Department of Thoracic Medicine

Department of Pathology

ETHICAL CLEARANCE: Clearance obtained

CONSENT: Individual written and informed consent.

ANALYSIS: STATISTICAL ANALYSIS

CONFLICT OF INTEREST : NIL

FINANCIAL SUPPORT: SELF

STATISTICAL ANALYSIS:

The information collected regarding all the selected cases were recorded in a master chart. Data analysis was done with statistical software SPSS.16 software and Sigma Stat 3.5 version.

Using this software, mean, standard deviation and p-value were calculated through one way ANOVA, Chi-square test .P- value of < 0.05 was taken as significant.

Pearson correlation coefficient was used to find correlation between 2 variables.

OBSERVATION AND RESULTS

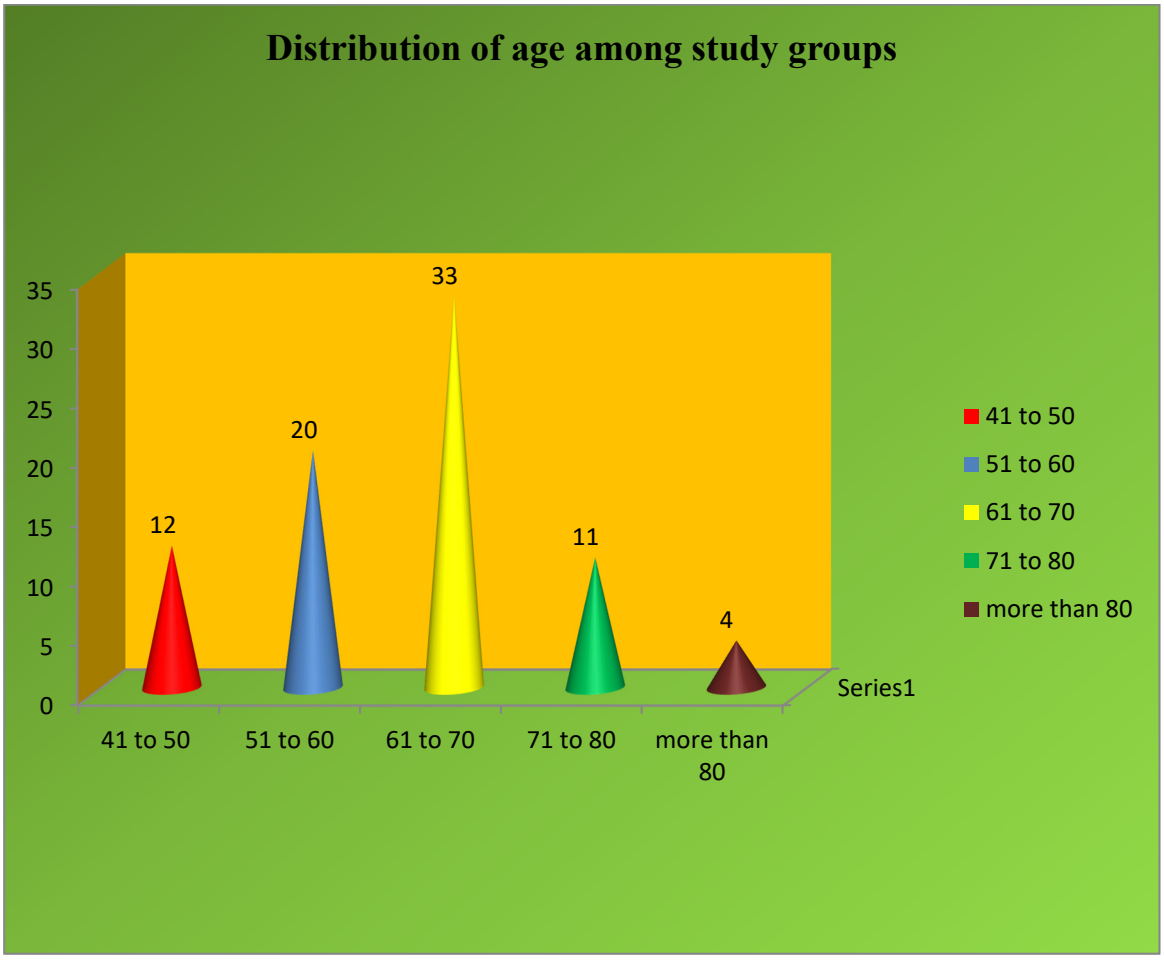
OBSERVATIONS AND RESULTS

AGE DISTRIBUTION IN COPD

In our study including 80 cases,

- 30.5% cases (12) between 41-50 years.
- 31.5% cases (20) between 51-60 years.
- 17.5% cases (33) between 61-70 years.
- 16% cases (11) between 71-80 years.
- 4.5% cases (4) between >80 years.

Age	No. of cases	Percentage
41 - 50	12	15
51 - 60	20	25
61 - 70	33	41.2
71 - 80	11	13.8
> 80	4	5


Distribution of age among study groups
35
30
25
20
15
10
5
0
12
20
33
11
4
41 to 50
51 to 60
61 to 70
71 to 80
more than 80
Series1
41 to 50
51 to 60
61 to 70
71 to 80
more than 80

SEX DISTRIBUTION

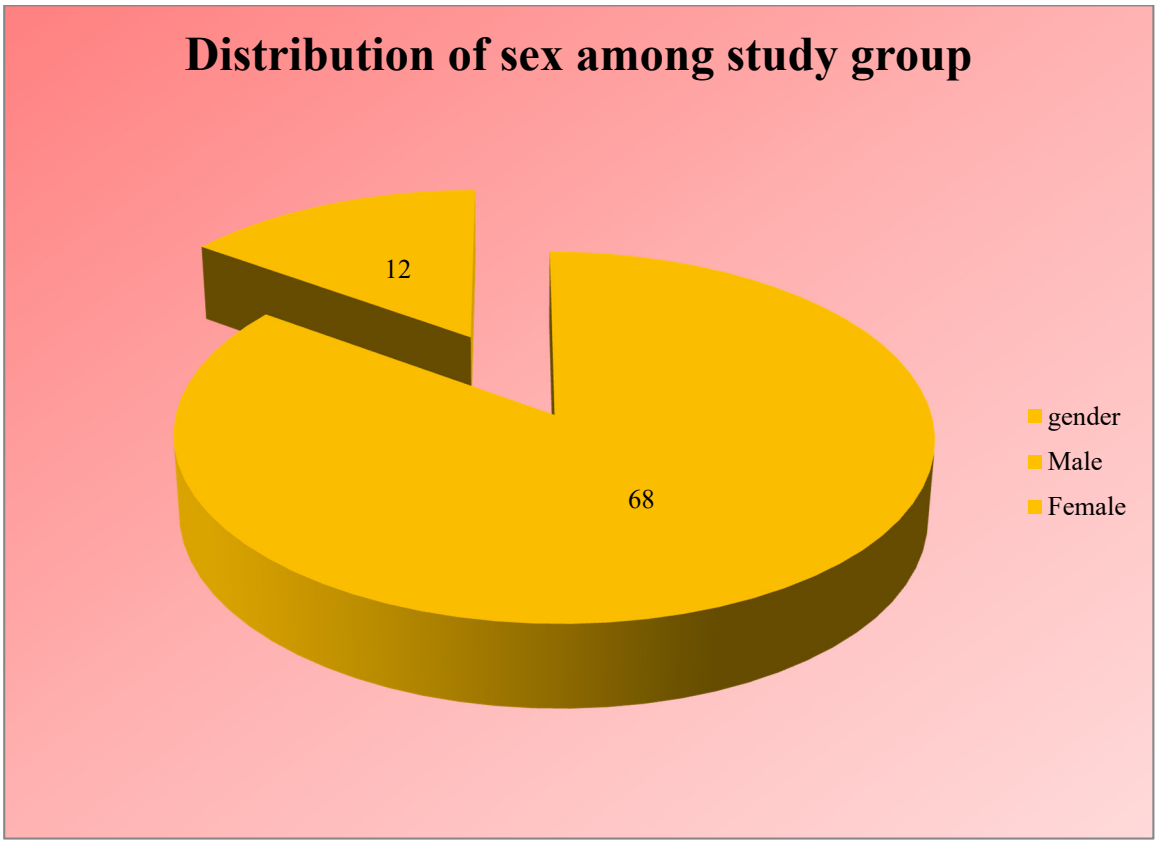
Sex	No. of cases	Percentage
Male	68	85
Female	12	15

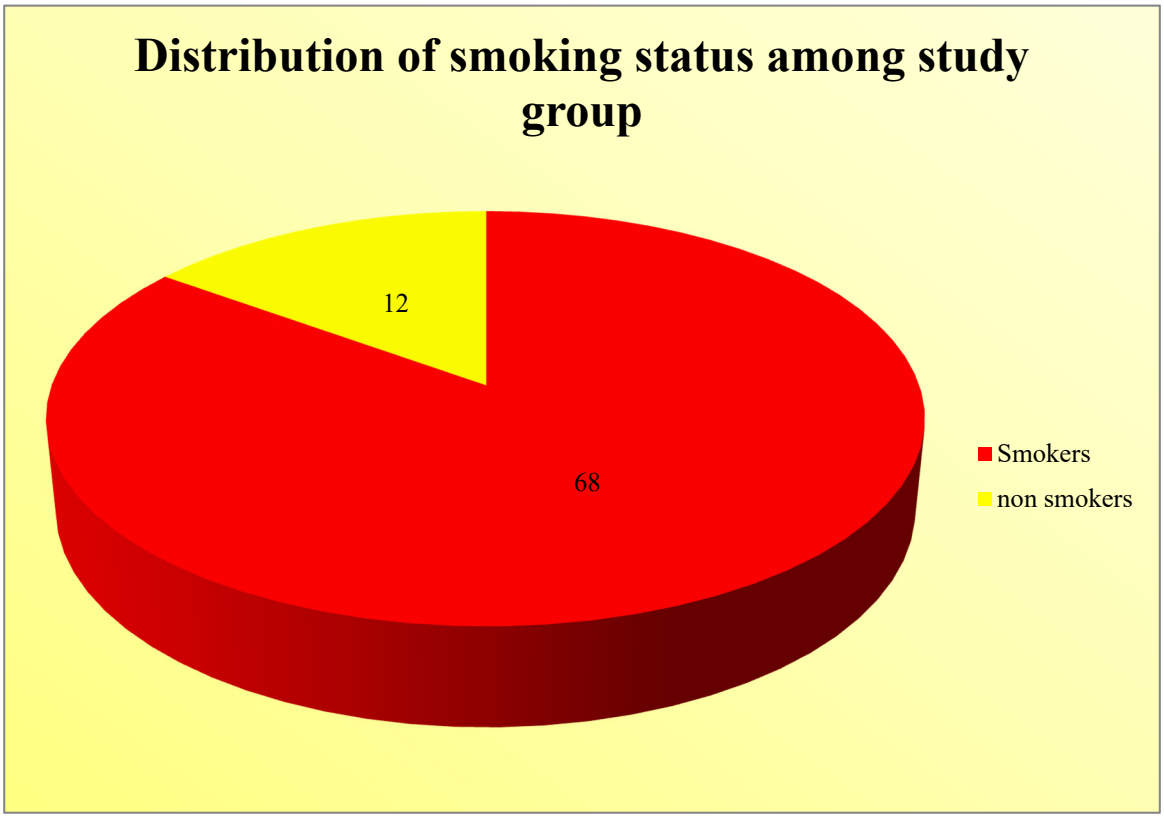
COPD is more common in males.Our study also reveals the same. The results of our study are as follows:

- Males involved are 68 (85%);
- Females involved are 12(15%).

There is increased incidence of COPD in smokers. It is well documented in many studies and theories. Our study also clearly proves the fact of increased incidence in smokers.

In our study, all the males were smokers and all the females were nonsmokers.


Distribution of sex among study group
12
68
gender
Male
Female


Distribution of smoking status among study group
12
68
Smokers
non smokers

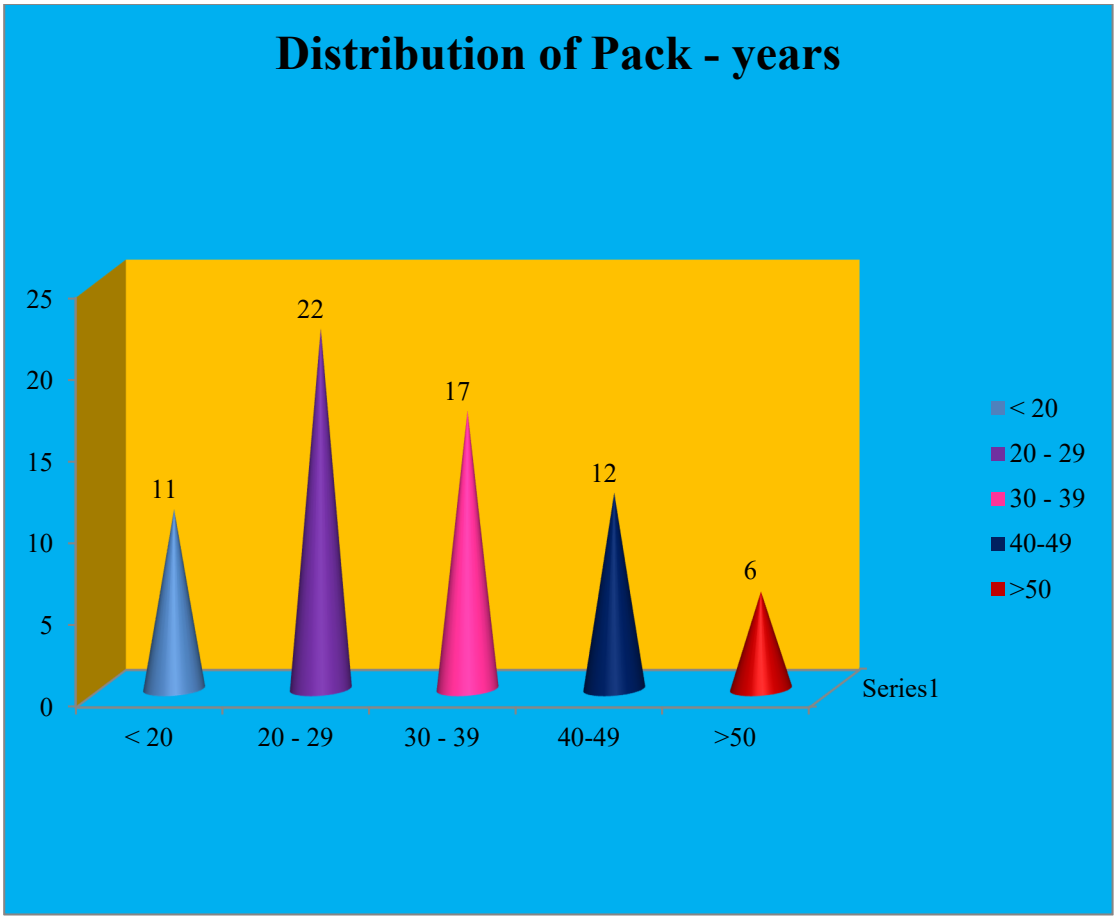
SMOKING AND COPD

In our study 68 cases (85%) out of 80 cases were smokers. Out of this

- 16.2% cases had < 20 pack years.
- 32.4% cases had 20-29 pack years.
- 25% cases had 30-39 pack years.
- 17.6% cases had 40-49 pack years.
- 8.8% cases had >50 pack years

	Cases	Percentage
Smokers	68	85
Non smokers	12	15

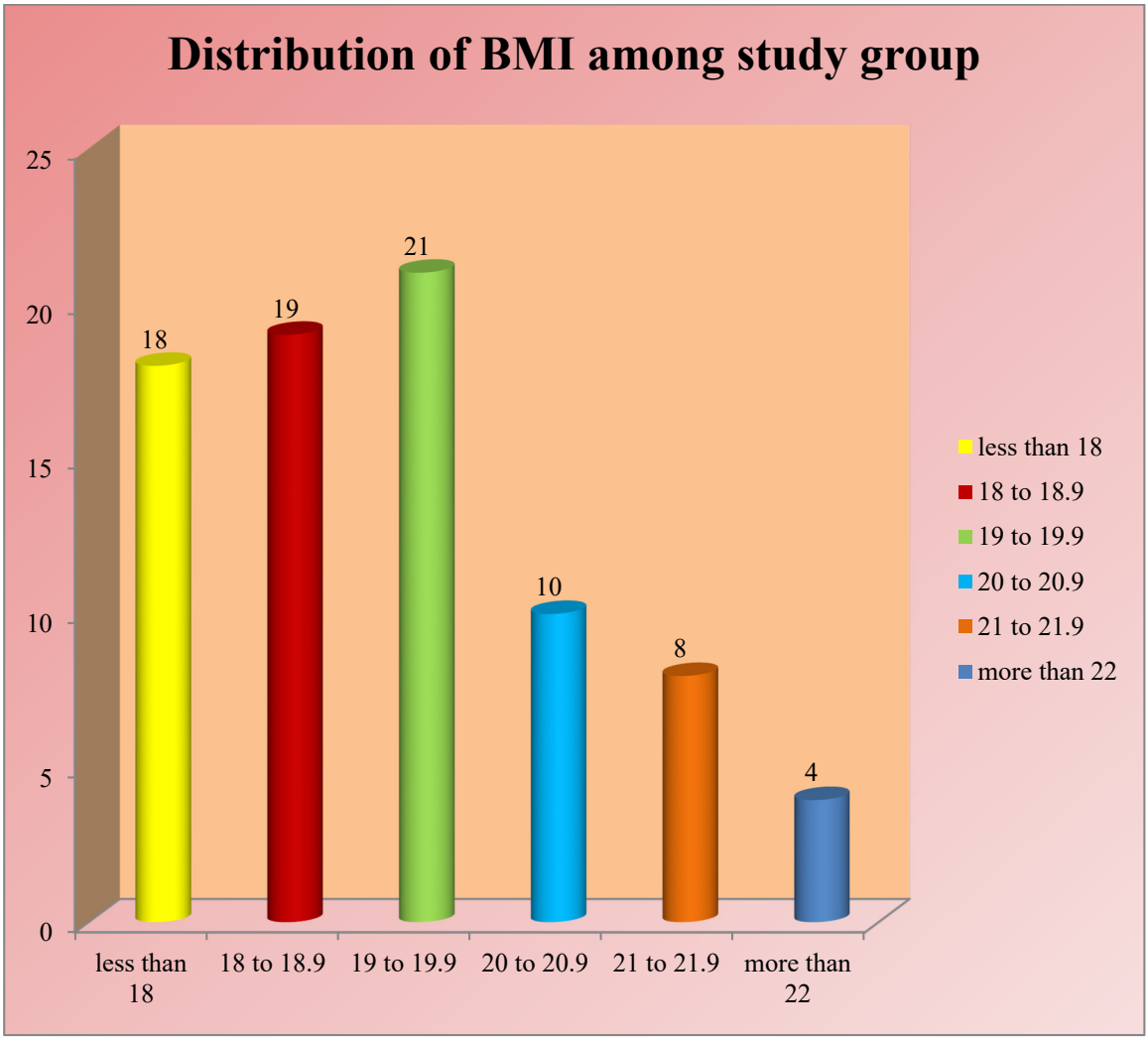
Smokers (Pack years)	No. of cases	Percentage
< 20	11	16.2
20 - 29	22	32.4
30 - 39	17	25
40-49	12	17.6
>50	6	8.8



BMI and COPD

In our study,

BMI	No. of cases	Percentage
<18	18	22.5
18-18.9	19	23.8
19-19.9	21	26.2
20-20.9	10	12.5
21-21.9	8	10
>22	4	5


Distribution of BMI among study group
25
20
15
10
5
0
18
19
21
10
8
4
less than 18
18 to 18.9
19 to 19.9
20 to 20.9
21 to 21.9
more than 22
less than 18
18 to 18.9
19 to 19.9
20 to 20.9
21 to 21.9
more than 22

Relation between BMI and NLR

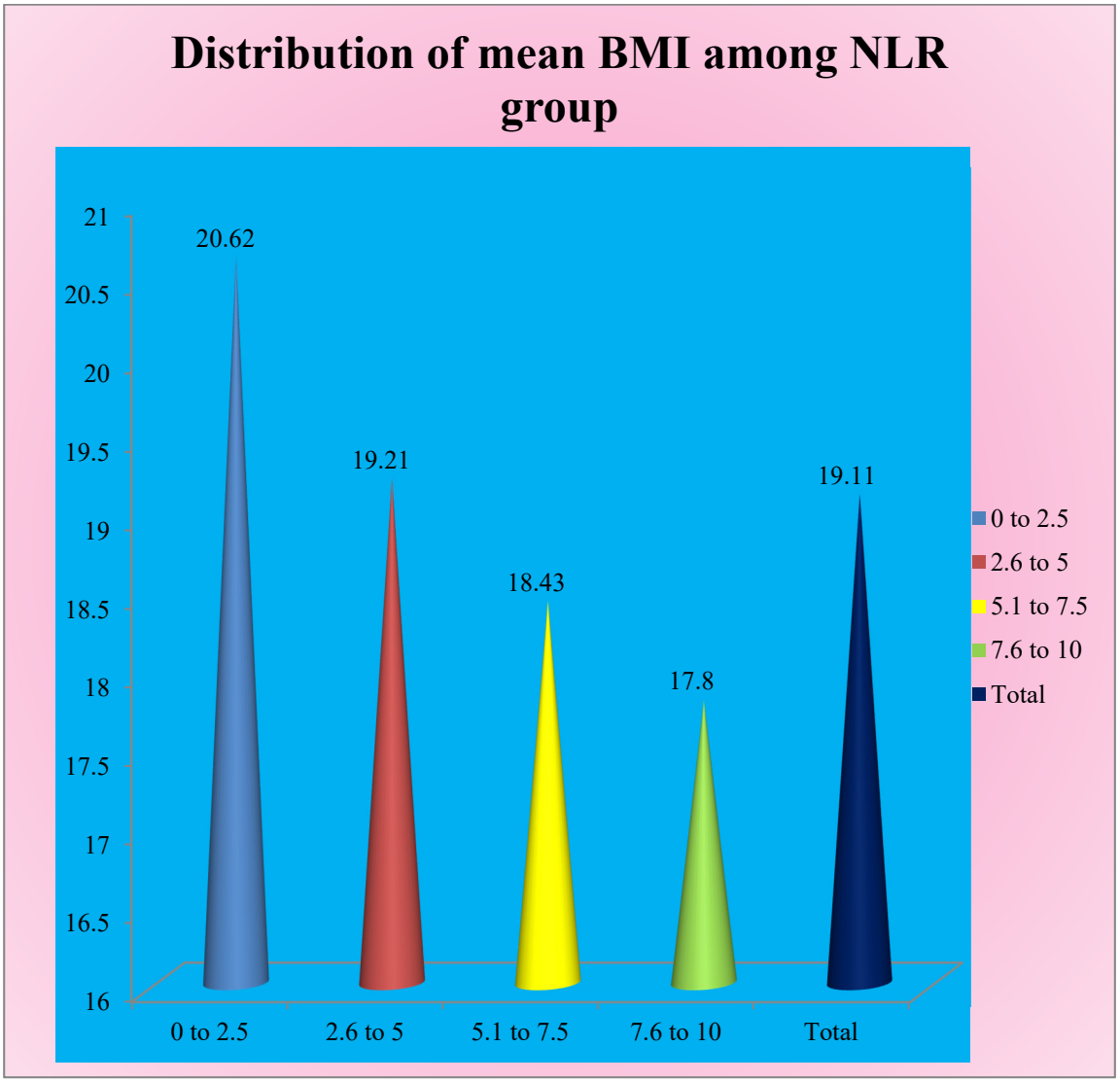
The relation between mean BMI and NLR as also assessed in our study.

NLR groups	Mean BMI
0 to 2.5	20.62
2.6 to 5	19.21
5.1 to 7.5	18.43
7.6 to 10	17.8

As NLR increases, mean BMI decreases.

NLR is associated with disease severity.

Hence, it can be inferred that lower BMI is associated with severe disease and poor prognosis.


Distribution of mean BMI among NLR group
21
20.5
20
19.5
19
18.5
18
17.5
17
16.5
16
20.62
19.21
18.43
17.8
19.11
0 to 2.5
2.6 to 5
5.1 to 7.5
7.6 to 10
Total

FEV1 % and COPD

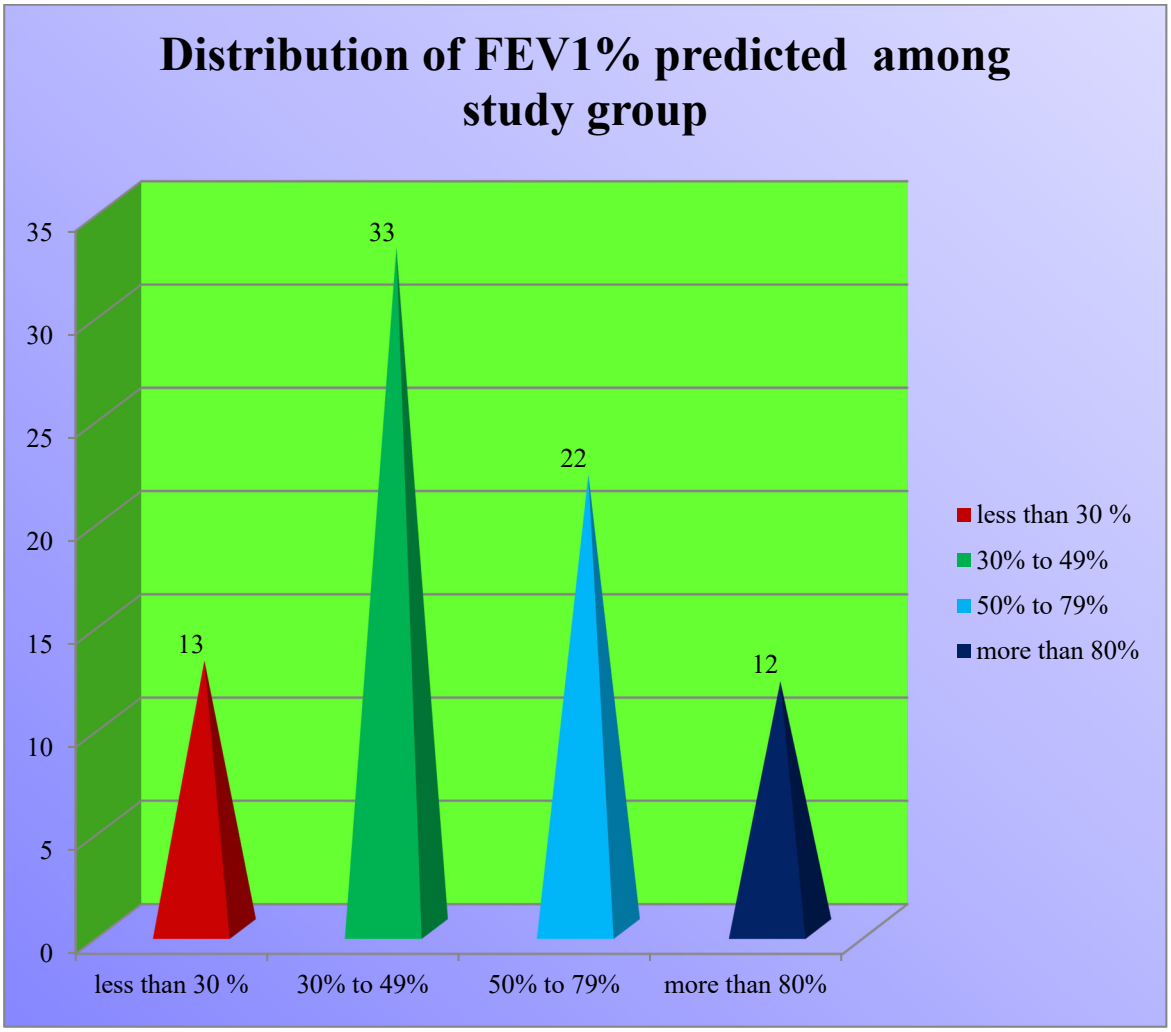
FEV1 < 30% (very severe) – 16.2% cases (13)

FEV1 30 – 49% (severe) – 41.2% cases (33)

FEV1 50 – 79% (moderate) – 27.5% cases (22)

FEV1 80% (mild) – 15% cases (12)

FEV1 %	No.of cases	Percentage
<30	13	16.2
30 – 49	33	41.2
50 -79	22	27.5
>80	12	15


Distribution of FEV1% predicted among study group
35
30
25
20
15
10
5
0
13
33
22
12
less than 30 %
30% to 49%
50% to 79%
more than 80%
less than 30 %
30% to 49%
50% to 79%
more than 80%

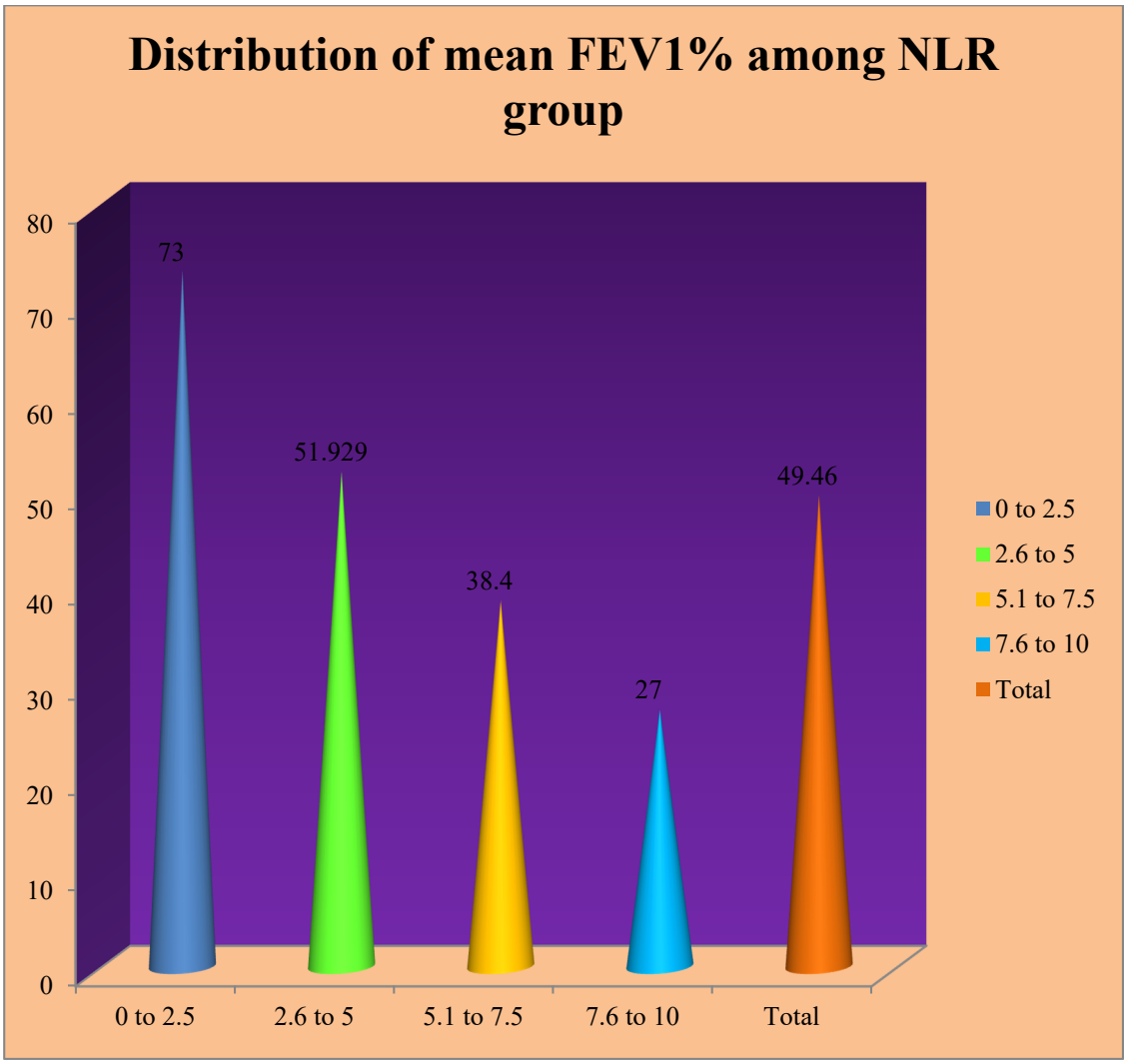
Relation between FEV1% and NLR

NLR	FEV1%(mean)
0 to 2.5	73
2.6 to 5.0	51.9
5.1 to 7.5	38.4
7.6 to 10	27

Shows significant p-value (0.001) using ANOVA test.

That is, FEV1% decreases, NLR increases

Ie. MORE severe the airflow obstruction (as evidenced by decreasing FEV1), Higher the NLR.


Distribution of mean FEV1% among NLR group
80
70
60
50
40
30
20
10
0
73
51.929
38.4
27
49.46
0 to 2.5
2.6 to 5
5.1 to 7.5
7.6 to 10
Total
0 to 2.5
2.6 to 5
5.1 to 7.5
7.6 to 10
Total

Modified medical research council scale(mMRC) and COPD

In our study,

2.5% cases had mMRC grade 0

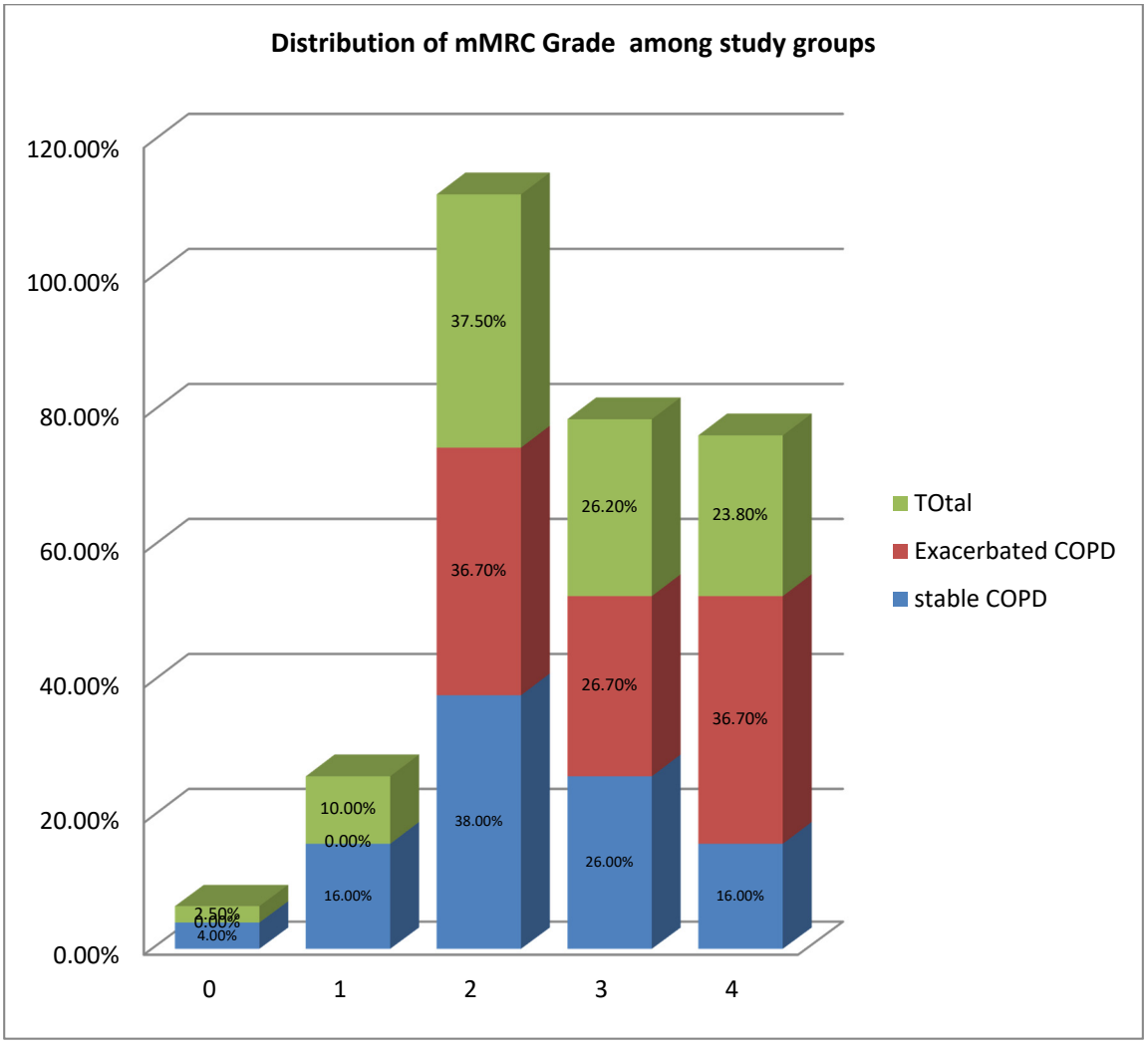
10% had mMRC grade 1

37.5% had mMRC grade 2

26.2% had mMRC grade 3

23.8% had mMRC grade 4

mMRC scale	No.of cases	Percentage
0	2	2.5
1	8	10
2	30	37.5
3	21	26.2
4	19	23.8


Distribution of mMRC Grade among study groups
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
0
1
2
3
4
37.50%
36.70%
26.20%
26.70%
23.80%
36.70%
10.00%
0.00%
16.00%
38.00%
26.00%
16.00%
2.50%
0.00%
4.00%
TOtal
Exacerbated COPD
stable COPD

Correlation between NLR and mMRC scale

Correlation between NL ratio grp and mMRC grade			**R value**	
Spearman's rho	NL_ratio_ grp and mMRC grade	Correlation Coefficient	0.825**	Positively correlated
		p value	.004	

The correlation coefficient between NLR and mMRC scale is 0.825.

Hence, there is a positive correlation between NLR and mMRC scale.

So, as the mMRC grade increases, NLR also increases.

6-MINUTE WALK DISTANCE AND STABLE COPD

6-minute walk test was performed among the stable COPD group. The results were as follows:

6 - minute walk distance (metre)	No.of cases	Percentage
>350	20	40
350-349	18	36
150-249	8	16
<149	4	8

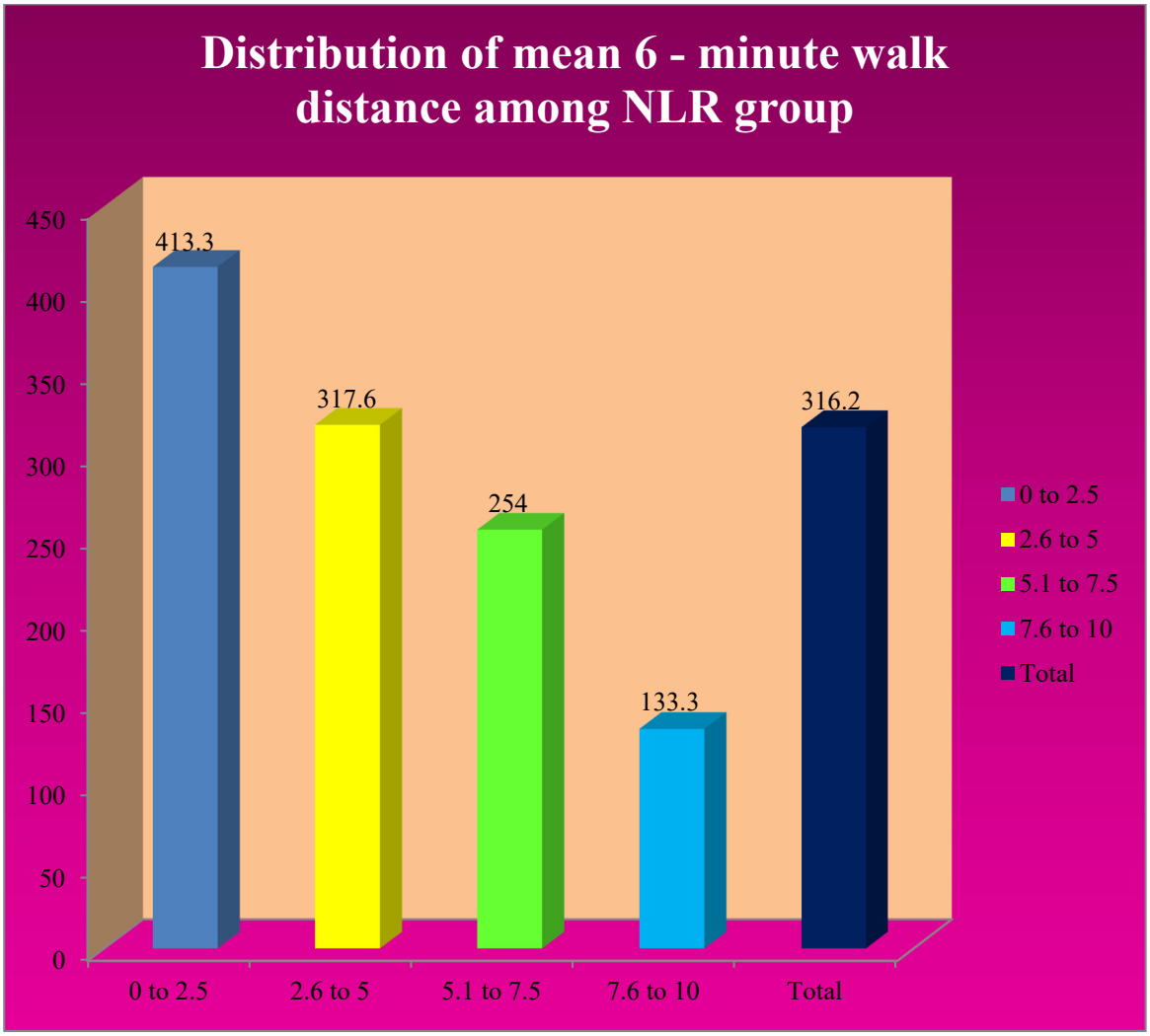
RELATION BETWEEN NLR AND 6-MINUTE WALK DISTANCE

NLR	6-minute walk distance
0 to 2.5	413.3
2.6 to 5	317.6
5.1 to 7.5	254
7.6 to 10	133.3

Shows significant p value (0.001) by ANOVA test

AS NLR increases, the mean 6-minute walking distance decreases.

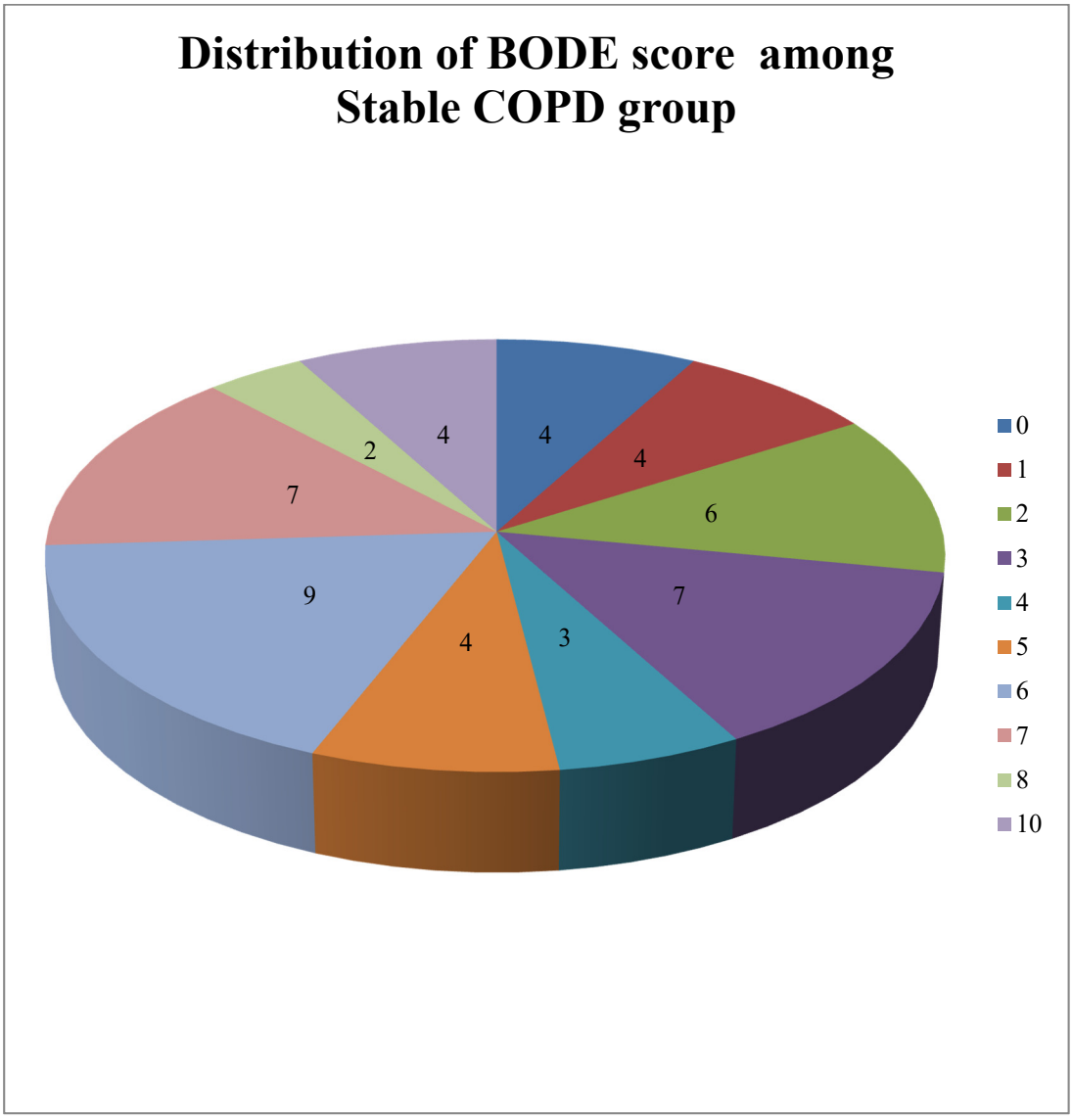
NLR is inversely correlated with 6-minute walk distance.


Distribution of mean 6 - minute walk distance among NLR group
450
400
350
300
250
200
150
100
50
0
413.3
317.6
254
133.3
316.2
0 to 2.5
2.6 to 5
5.1 to 7.5
7.6 to 10
Total
0 to 2.5
2.6 to 5
5.1 to 7.5
7.6 to 10
Total

BODE SCORE

The BODE SCORE was calculated for the stable COPD group. The results are as follows:

BODE SCORE	No.Of Cases
0	4
1	4
2	6
3	7
4	3
5	4
6	9
7	7
8	2
9	0
10	4


Distribution of BODE score among Stable COPD group
4
4
6
7
3
4
9
7
2
4
0
1
2
3
4
5
6
7
8
10

NEUTROPHIL - LYMPHOCYTE RATIO (NLR)

		NL_ratio_grp				P value
		0 to 2.5	**2.6 to 5**	**5.1 to 7.5**	**7.6 to 10**	
Stable COPD	frequency (n)	15	17	15	3	0.01
	% within	30.0%	34.0%	30.0%	6.0%	
Exacerbated COPD	frequency (n)	2	10	15	3	
	% within	6.7%	33.3%	50.0%	10.0%	
Total	frequency (n)	17	27	30	6	
	% within	21.2%	33.8%	37.5%	7.5%	

In our study,

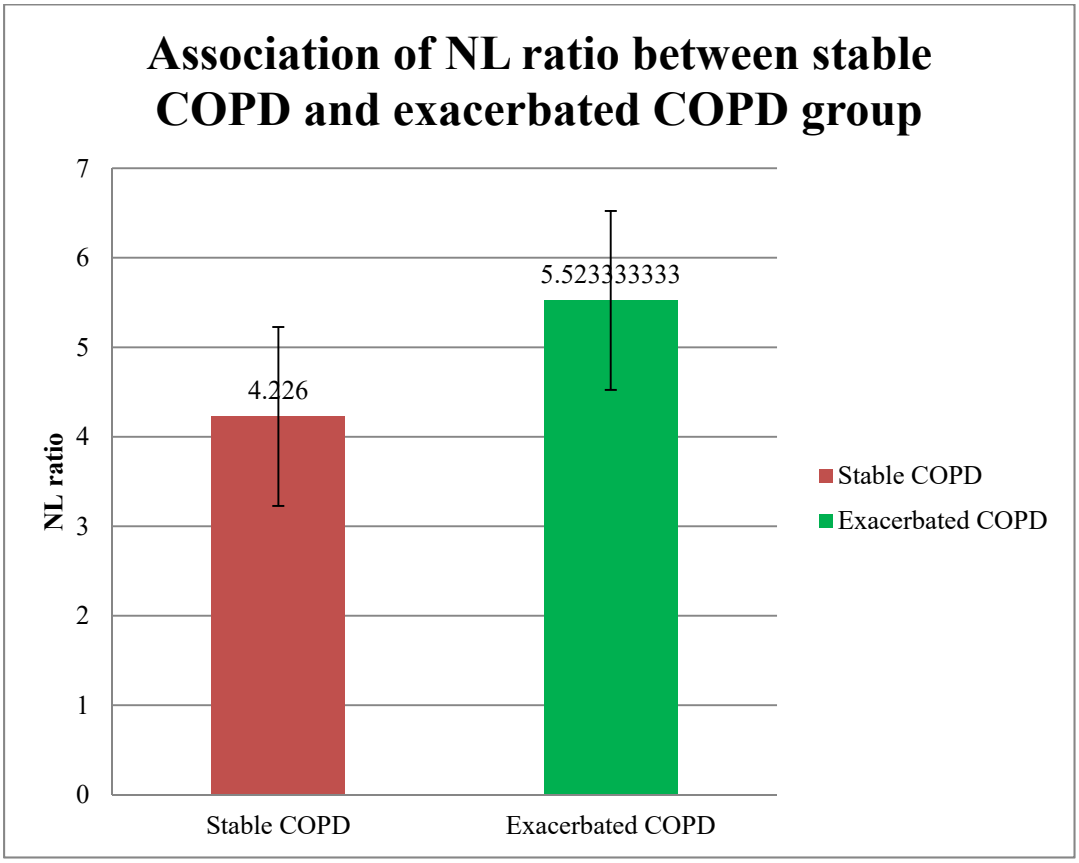
21.2% had NLR between 0 to 2.5

33.8% had NLR between 2.6 to 5

37.5% had NLR between 5.1 to 7.5

7.5 % had NLR between 7.6 to 10

Comparison of NLR between stable COPD and COPD exacerbation group



Comparison of NLR between the stable and exacerbated COPD was done using the unpaired t-test and was found to be statistically significant (p value-0.008)

It is found that NLR is higher among the COPD Exacerbation group as compared to stable COPD.

Hence, it can be inferred that NLR can be used as a marker of COPD exacerbation.

NLR and BODE

		BODE score				Total	P value
	NLR	**0 to 2**	**3 to 5**	**6 to 8**	**9 to 10**		
0 to 2.5	Count	13	2	0	0	15	0.0001
	% within bode	92.9%	14.3%	.0%	.0%	30.0%	
2.6 to 5	Count	1	11	5	0	17	
	% within bode	7.1%	78.6%	27.8%	.0%	34.0%	
5.1 to 7.5	Count	0	1	13	1	15	
	% within bode	.0%	7.1%	72.2%	25.0%	30.0%	
7.6 to 10	Count	0	0	0	3	3	
	% within bode	.0%	.0%	.0%	75.0%	6.0%	
Total	Count	14	14	18	4	50	
	% within bode	100.0%	100.0%	100.0%	100.0%	100.0%	

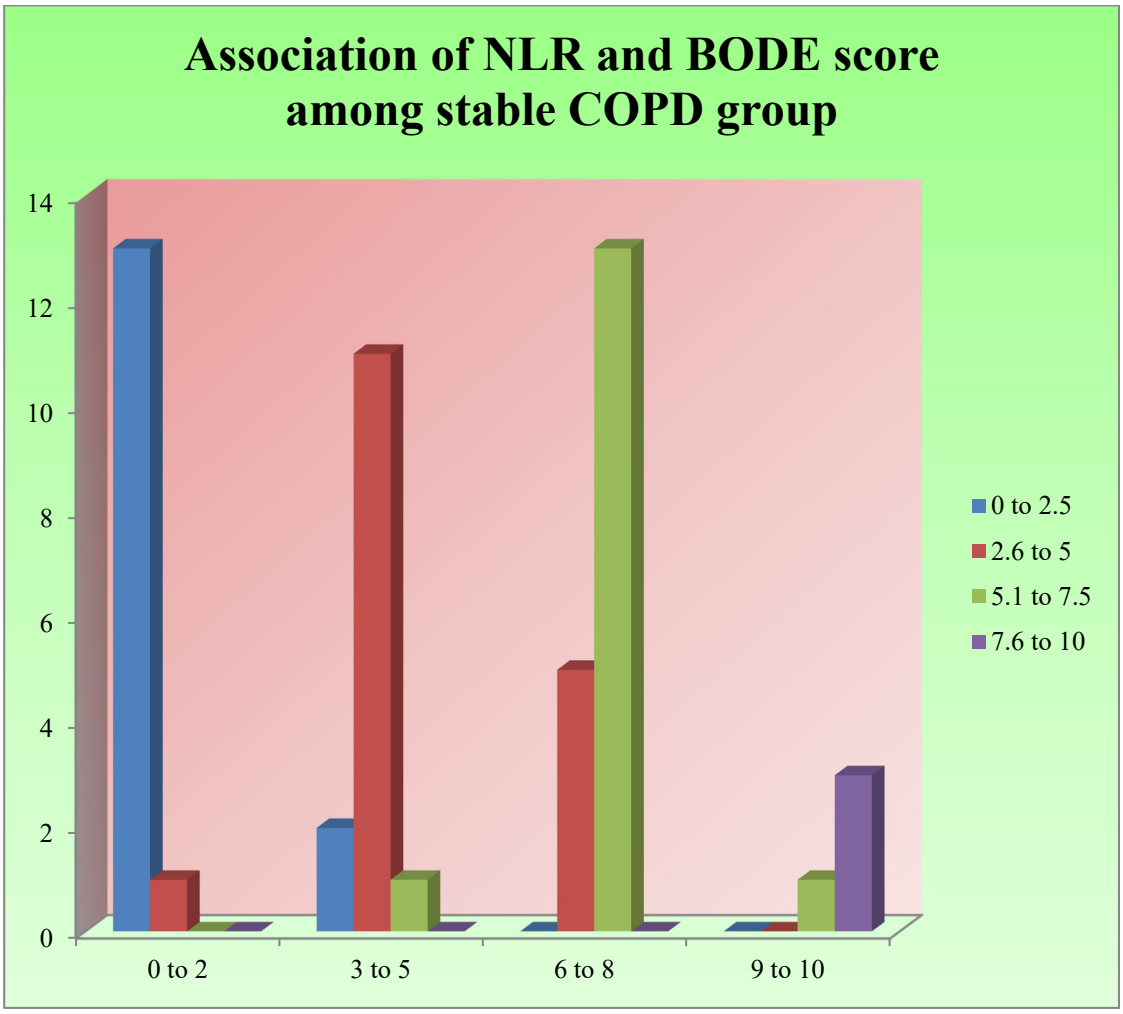
* Shows significant p value chi square test

As NLR increases, BODE score increases

There is positive correlation between BODE and NLR

Correlation coefficient +0.916

Hence, NLR can be used to assess the disease severity as it correlates with BODE score.


Association of NLR and BODE score among stable COPD group
14
12
10
8
6
4
2
0
0 to 2
3 to 5
6 to 8
9 to 10
0 to 2.5
2.6 to 5
5.1 to 7.5
7.6 to 10

DISCUSSION

DISCUSSION

In our study, all the cases are aged above 40 years. Maximum number of cases(33) fell between 61-70 years. The findings of our study are similar to our earlier knowledge since COPD is a disease affecting elderly persons.

In our study,85% of patients were males and 15% were females. The higher prevalence in males is probably due to the higher prevalence of smoking in males.

All the males in our study are smokers and all the females are nonsmokers. Cigarette smoking is a major etiological risk factor for COPD. In our study, it is proved beyond doubt that there is a significant association between smoking and COPD.

Our study shows that a low BMI is associated with a high NLR and severe disease. Hallin et al[10] in 2006 reported that low BMI and weight change ere related to a poor prognosis in COPD. Pouw et al[11] in 2000 reported that low BMI is a risk factor for unplanned readmission. Hence, our results are in accord with the previous studies that further efforts are needed to improve the nutritional status in patients with COPD.

In our study, majority of patients[41.2%] came under GOLD stage 3.As FEV1% decreases, NLR increases.ie.as the severity of airflow obstruction increases, NLR increases. Thus, NLR indirectly reflects the extent of airflow obstruction. A possible underlying mechanism is that activated neutrophils cause tissue destruction in lungs by releasing oxygen radicals and proteolytic enzymes which results in emphysema. Emphysematous changes may lead to small airway obstruction. Yasar et al[22] in 2015 have detected a negative correlation between NLR and FEV1%.Our study has also yielded the same.

In our study, majority (37.5%) had a mMRC dyspnoea scale of 2.We obtained a positive correlation (0.825) between NLR and mMRC.

The relation between NLR and 6-minute walk distance as also assessed. As NLR increases, the 6-minute walk distance reduced. Thus, NLR and 6-minute walk distance are inversely correlated. Ryuko et al in 2016 has also yielded similar results.

The relation between NLR and BODE scores were assessed. There is a positive correlation between NLR and BODE score with a p-value of 0.0001 and correlation coefficient of 0.916.

Celli et al [24] in 2004 constructed the BODE score to predict the risk of death among patients with COPD. Seung et al in 2016 has

demonstrated the association between NLR and BODE score as well as with individual components of BODE. They showed significant correlation between NLR and BODE score, mMRC and 6 minute walk distance whereas no correlation was identified between NLR and BMI or FEV1.But our study contradicts their study in that NLR have been correlated well with BODE score as well as with mMRC,6-minute walk distance, BMI and FEV1%.

Another important observation was that NLR was higher among the COPD exacerbation group as compared to the stable COPD group with a p value of 0.008.Hence, it can be inferred that NLR can be used as a marker of COPD exacerbation.

Thus, NLR can be used as a prognostic indicator and determinant of severity of COPD is well proved statistically in our study.

LIMITATIONS

1. Relatively small sample size
2. BODE score could only be assessed in the stable COPD group
3. It is a cross-sectional study, hence control groups could not be involved.

CONCLUSION

CONCLUSION

- NLR was significantly higher in COPD exacerbation compared to patients with stable COPD
- NLR correlated with the disease severity as it has positive correlation with BODE score
- COPD is more prevalent among smokers and male sex
- Low BMI is a risk factor for severe disease
- NLR correlated with the severity of airflow obstruction
- NLR has a positive correlation with mMRC scale
- NLR correlated inversely with 6-minutre walk distance
- Thus, NLR correlated inversely with BMI,FEV1 %,6-minute walk distance and has a positive correlation with mMRC scale.
- So, NLR has got significant correlation with BODE score as well as all the individual parameters in BODE score.
- Since NLR correlates well with the disease severity in COPD, it should be used as a routine predictive marker of mortality in all patients with COPD since it is simple, cost-effective and can be obtained from a routine complete blood count in comparison to calculating the BODE score which is cumbersome.

ANNEXURES

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ABBREVIATIONS

NLR	-	Neutrophil Lymphocyte Ratio
FEV1	-	Forced expiratory volume
FVC	-	Forced vital capacity
COPD	-	Chronic obstructive pulmonary disease
PFT	-	Pulmonary function test
GOLD	-	Global guidelines for obstructive pulmonary diseases

PROFORMA

Personal details

Age/Sex: Occupation: OP/IP No.:

Presenting complaints:

Grade of dyspnea (MMRC scale)

Past history:

Total duration of illness

H/o DM, HIV, PT, HT, CKD, CVD, COPD etc

Current smoker/Ex-smoker Pack-years

Clinical examination

General examination:

Height- Weight- BMI-

Consciousness, Pallor, Jaundice, Cyanosis, Clubbing, Lymphadenopathy,

Pedal edema

Vitals: PR _____ BP_____ Temperature_____ RR_____ SpO2___

Systemic examination:

Respiratory system-

CVS: Abd: CNS:

Pulmonary Function Tests:

FEV1- Post-bronchodilator FEV1-

FVC-

FEV1/FVC- **GOLD STAGE-**

Laboratory investigations

Total Leucocyte count:

Differential count of neutrophils

Differential count of lymphocytes

Neutrophil-to-Lymphocyte ratio

MASTER CHART

STABLE COPD

S. NO	AGE	SEX	BMI	SMOKING STATUS (PACK-YEARS)	FEV1 % PREDICTED	GOLD STAGE	mMRC GRADE	6 MINUTE WALK DISTANCE(m)	BODE SCORE	NL RATIO
1	62	M	20.3	40	34	3	2	280	6	2.8
2	69	M	18.9	20	28	4	2	300	6	3.4
3	55	M	20.5	25	36	3	2	280	5	3.8
4	59	M	21	30	52	2	2	300	4	2.6
5	61	M	19.2	30	26	4	4	140	10	8.2
6	64	F	19.6	NIL	46	3	2	300	5	4.6
7	65	M	18.5	20	38	3	3	180	7	6.2
8	67	M	22.1	15	52	2	2	380	2	2.1
9	58	M	21.3	10	32	3	3	280	6	5.5
10	57	F	20.6	NIL	82	1	1	400	1	1.8
11	66	M	19.4	25	56	2	3	260	4	3.6
12	63	M	19.8	25	64	2	1	380	2	1.9
13	62	M	18.6	45	44	3	1	360	3	4.8
14	56	F	19.5	NIL	73	2	2	400	2	2.2
15	68	M	22	30	84	1	1	400	0	1.8
16	63	M	21.1	15	38	3	2	370	3	2.9
17	61	M	23	20	80	1	1	500	0	2
18	60	M	20.1	15	86	1	2	440	2	3.4
19	65	M	18.3	20	37	3	2	300	5	5.3
20	68	M	18.6	25	26	4	4	120	10	7.4
21	66	M	16.2	30	58	2	2	340	4	3.6
22	50	M	19.4	35	36	3	3	200	7	7.4
23	59	F	19.8	NIL	56	2	2	380	3	2.8
24	76	M	21.5	30	42	3	3	260	6	5.2
25	66	M	19.1	35	24	4	3	240	7	5.8
26	54	M	19.2	25	56	2	2	420	3	2.8
27	65	M	17.6	35	36	3	3	260	6	5.6
28	43	F	18.2	NIL	24	4	4	140	10	8.8
29	58	M	20.1	35	54	2	2	380	3	2.4
30	45	M	18.4	15	34	3	2	220	7	7.1
31	66	M	20.4	30	58	2	2	360	3	3.6
32	59	M	19.4	35	38	3	4	280	7	6.8
33	68	M	18.4	25	46	3	3	300	6	4.4
34	45	M	20.5	10	80	1	1	380	1	1.8
35	56	M	18.7	20	48	3	3	230	7	6.4
36	74	M	20.5	40	66	2	3	190	5	4.8

37	47	M	19.6	25	54	2	3	220	6	4.8
38	54	F	19.7	NIL	72	2	1	440	1	1.6
39	77	M	20.6	25	68	2	2	320	3	2.2
40	47	M	17.8	45	34	3	4	120	10	8.6
41	67	M	19.4	20	70	2	2	420	2	2
42	81	M	17.2	60	22	4	4	260	8	7.1
43	68	F	19.4	NIL	38	3	3	300	6	4.3
44	46	M	18.6	35	76	2	2	380	2	1.9
45	52	M	22.3	15	82	1	0	500	0	1.2
46	57	F	19.6	NIL	48	3	3	320	6	5.9
47	48	M	21.6	20	84	1	1	440	0	1.1
48	65	M	17.1	30	48	3	4	340	7	6.2
49	68	M	19.8	25	44	3	4	320	8	7.4
50	58	M	18.4	15	80	1	0	480	1	1.4

COPD EXACERBATION

SL. NO	AGE	SEX	BMI	SMOKING STATUS (PACK-YEARS)	FEV1 % PREDICTED	GOLD STAGE	mMRC GRADE	NL RATIO
1	72	M	16.9	40	28	4	4	4.3
2	70	M	16.3	20	44	3	4	5.6
3	72	M	16.4	25	32	3	2	3.1
4	78	M	17.6	30	18	4	3	5.8
5	65	M	18	30	36	3	4	6.4
6	63	M	18.4	20	80	1	2	4.2
7	64	M	20	40	60	2	2	4.1
8	68	F	18.3	NIL	66	2	2	3.5
9	54	M	19.6	48	38	3	4	6.2
10	60	M	18.2	50	64	2	3	5.9
11	68	M	17.4	45	46	3	2	6.1
12	79	M	17.2	55	40	3	4	7.2
13	83	M	16.8	60	26	4	3	8.6
14	50	M	16.9	48	40	3	3	7.3
15	54	F	18	NIL	24	4	4	8.1
16	72	M	19.6	40	56	2	2	6.7
17	74	M	19.6	50	64	2	3	5.6
18	70	M	21	15	82	1	2	4.3
19	68	M	18	60	34	3	4	6.8
20	68	M	17.4	20	86	1	2	3.9
21	64	M	17.2	25	26	4	4	7.4
22	44	F	18.9	NIL	36	3	3	2.8
23	52	M	19.2	25	42	3	2	4.6
24	82	M	21.4	15	58	2	2	1.9
25	76	M	17.5	45	22	4	4	7.2
26	48	F	19.4	NIL	38	3	3	4.4
27	76	M	17.1	35	36	3	4	6.9
28	58	M	18.4	45	35	3	3	6.4
29	49	M	21.1	18	82	1	2	1.8
30	85	M	16.8	30	28	4	4	8.6

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.Gokul prasannan
Course	:	PG in MD., General Medicine
Period of Study	:	2015-2018
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Neutrophil-Lymphocyte Ratio as a marker of disease severity and exacerbation in COPD"
Ethical Committee as on	:	27.07.2017

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary | Chairman | Dean/Convenor

Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc.(Hon)
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Urkund Analysis Result

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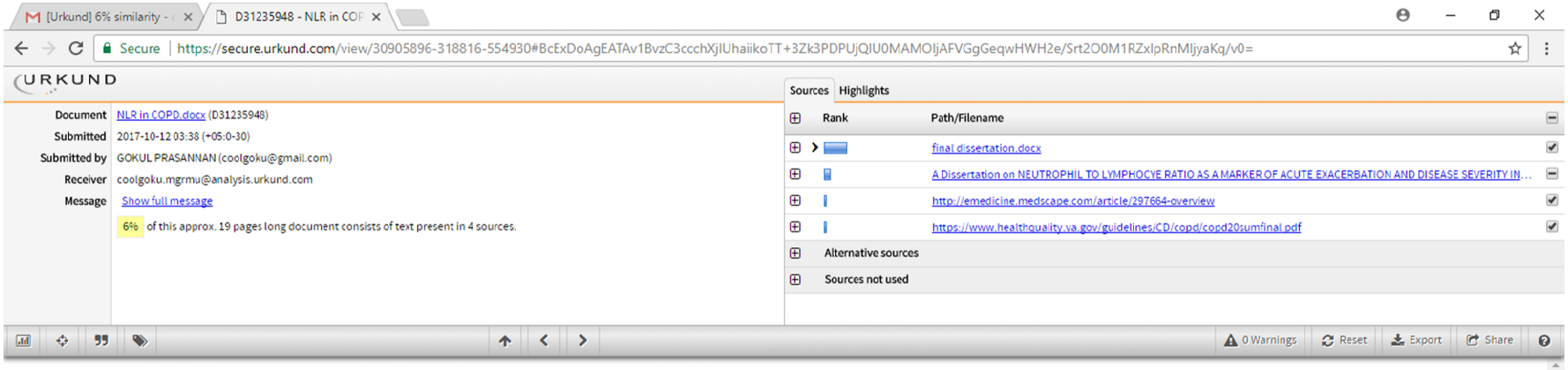

NEUTROPHIL-LYMPHOCYTE RATIO AS A MARKER OF DISEASE SEVERITY AND EXACERBATION IN COPD

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